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Proefschrift

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In memory of my father
To my mother

TABLE OF CONTENTS

1. GENERAL INTRODUCTION

1.1 General considerations to the aetiology of hypertension

Chronic hypertension in man may be divided into primary and secondary forms. The latter includes hypertension of known causes such as coarctation of the aorta, pheochromocytoma, primary aldosteronism, chronic glomerulonephritis, and renovascular hypertension. However, more than 90% of patients with elevated blood pressure, have primary or essential hypertension. The term "essential" refers to the unknown cause of the disease. The disease is wide-spread with a prevalence of 15-20% in Western countries (Society of Actuaries, 1980; Epstein, 1983; Julius and Hansson, 1983). No major cause has been demonstrated for essential hypertension and its aetiology has outgrown the image of being a single homogeneous disease. Current hypotheses concerning the heterogeneous pathophysiology of essential hypertension can be summarized as follows:

1. It is a disorder of regulation in which a complex interplay of short- and long-term blood pressure control mechanisms are involved (e.g. endocrine, neurogenic, renal and vascular mechanisms). The disorder of regulation ultimately results in an elevated arterial blood pressure associated with an increased peripheral resistance.
2. Essential hypertension encompasses a variety of specific but still undefined pathophysiological disturbances, on the long run increasing resistance and thus blood pressure.
3. Essential hypertension cannot be regarded as a distinct entity because the distribution curves of blood pressure in the general population are smooth and there is no dividing line between normal and elevated blood pressure.

The multifacetted nature of the problem is reflected by the fact that a hypertensive state can be reproduced in a whole array of experimental models (e.g. dietary, hormonal, renal, neurogenic, or genetic selection). Each of these modalities can in turn be modified by a range of interventions so that the extensive data on the subject matter available in literature are difficult to synthetize into some

generalized scheme. According to Guyton (1986), two main extreme mechanisms related to the aetiology of hypertension can be distinguished. Hypertension can be the result of either fluid retention by the kidneys or by activation of vascular constrictor mechanisms. Most forms of experimental hypertension are mixed forms and it is yet not fully clarified to what extent each factor contributes to the increased blood pressure.

There is an important genetic component in human hypertension (Rapp, 1983). It has long been recognized that essential hypertension tends to occur in families. This tendency is polygenic rather than related to a single gene (Pickering, 1968). Although available evidence is difficult to quantify, it indicates that the contribution of inheritance is probably between one-third and two-thirds of the whole. The inheritance of (spontaneous) hypertension in the Okamoto-Aoki strains of the spontaneously hypertensive rat (SHR) is also polygenic, even though the number of genes involved may be relatively small (Tanase et al, 1972; Harrap, 1986). The SHR strain serves as an appropriate genetic animal model of human essential hypertension and therefore the present thesis will be limited to spontaneous hypertension in rats (Rapp, 1983).

Basically, two hypotheses have been put forward to explain the aetiology of spontaneous hypertension either related to the dominant role of the kidneys or to primary vascular changes. Before assessing the relationship of microvascular changes to the aetiology of spontaneous hypertension, some dominant blood pressure control mechanisms will be discussed for reasons of completeness. Most of them have been shown to be involved in the vasomotor activities of the microcirculation. Each mechanism will be discussed briefly from the perspective of the macrocirculation. In the second part of this introduction, the (micro)vascular dynamics in spontaneous hypertension will be discussed.

1.2 The pivotal role of the kidneys in the onset of spontaneous hypertension

No specific pathogenic mechanism has yet been found for essen-

tial hypertension in man nor in several animal models for hypertension. The dominance of the kidneys in the induction and maintenance of several forms of hypertension is well-known. Kidney transplantation from normotensive donor rats into rats of the Milan hypertensive strain resulted in complete reversal of blood pressure (Bianchi et al, 1973, 1974). This has also been demonstrated for the spontaneously hypertensive rat and the Dahl-hypertensive rat (Dahl et al, 1974). Conversely, blood pressure increased in normotensive rats after they received kidneys from prehypertensive or from hypertensive animals (Fox et al, 1976; Tobian, 1983).

1.2.1 Nervous influences on the kidney

Much attention has been paid to the role of the sympathetic nervous system in the aetiology of hypertension. An enhanced general activity of the sympathetic nervous system can play an important role in the onset and maintenance of hypertension (Abboud, 1982, 1984; McCarty, 1986). The primary effect of an enhanced sympathetic activity would be to increase peripheral vascular resistance. It had been shown that stimulation of renal adrenergic mechanisms influences a variety of kidney functions. Following stimulation, renal arteriolar resistance (Block et al, 1952; Fink et al, 1978), renin release (Davis and Freeman, 1976) and tubular sodium reabsorption (Colindres and Gottschalk, 1978; DiBona, 1977) are reported to be increased. The thick ascending limb of the loop of Henle is under adrenergic control as well (DiBona and Sawin, 1982).

It has been argued that a rise in peripheral resistance is only the cause of hypertension, if the rise seen in vascular resistance includes the renal vasculature. The renal vascular bed is among the first to raise its resistance during the development of spontaneous hypertension (Evenwel et al, 1983). An enhanced sympathetic activity affects primarily renal afferent arterioles which show the highest sensitivity for noradrenaline as compared to efferent arterioles (Edwards, 1983). Furthermore, a sympathetically mediated increased proximal tubular reabsorption of Na^+ and water leads to fluid retention (DiBona, 1982; Kopp et al, 1987). Circulating blood volume and plasma volume will increase and due to the elevation of circulatory

filling pressures, the cardiac output will increase. The blood pressure urinary flow curve will shift to higher pressures as has been shown for the spontaneously hypertensive rat (Norman et al, 1978; Roman, 1986).

Other factors have to be included as well. A stressful environment and a high sodium intake increase the responsiveness of the sympathetic nervous system to neurogenic stimuli to a greater degree in animals with a genetic predisposition toward hypertension and these stimuli interact synergistically (Koepke and DiBona, 1985). In addition, high sodium may result in disinhibition of central sympathico-inhibitory neurons and lead to a hyperadrenergic state (Gavras, 1986) which is probably reinforced by an heritable abnormal sodium handling (Harrap, 1986) observed in the spontaneously hypertensive rat.

Several authors have shown that section of the efferent nerves prevents or delays the development of hypertension in several rat models (Liard, 1977; Kline et al, 1980; Winternitz et al, 1980; Katholi et al, 1980, 1982). In most models it has however no effect on the final hypertensive state. Blood pressure increases could not be completely blocked partly due to functional reinnervation or to structural changes of the vasculature. The role of sympathetic nerves remains controversial because hypertension develops equally rapidly in rats maintained on a high sodium diet whether the renal nerves are present or not (Norman et al, 1984). The delay in blood pressure increase was explained on the basis of the rats ability to retain sodium and water. It should be taken into account that crucial experiments still have to be performed because no selective method to eliminate the renal sympathetic nerves was employed in these studies and renal nerves in the rats consist of afferent as well as efferent fibers (Le Noble et al, 1985; Smits et al, 1985).

1.2.2 Afferent renal nerve activity

The role of afferent renal nerve activity may be involved in the maintenance of a high sympathetic tone. Kidneys contain both mechano-sensitive (Niijma, 1971, 1972) and chemo-sensitive receptors with a sensory input into the central nervous system (Recordati et al, 1980, 1981, 1982; Zanchetti et al, 1984). These nerves are involved in

reflex cardiovascular adjustments. Moreover, electrophysiological evidence is available for the existence of renorenal reflexes. Short-term activation of afferent renal nerves in dogs or rats by means of chemical stimulation by substances like adenosine or bradykinin (Smits and Brody, 1984; Katholi, 1984, 1985) leads to increment of blood pressure and heart rate. Chronic stimulation of the renal afferent nerves could lead to increases in overall sympathetic activity as was shown in two-kidney, one-clip Goldblatt rats (Katholi et al, 1982).

In spontaneous hypertension, afferent renal nerves may be part of a still unknown vasopressor mechanism (Kleinjans et al, 1984). Although ablation of renal nerves prevents blood pressure to increase following pharmacological stimulation of renal afferent sensors, no attenuation in the development of high blood pressure was seen in SHR in which afferent nerves were selectively eliminated by means of dorsal rhizotomy (Janssen et al, 1987). Thus the significance of afferent renal nerves may be linked to short-term cardiovascular adjustments rather than to a state of a chronically elevated blood pressure.

1.2.3 Hormonal influences

Several hormones are involved in renal excretory function. Apart from the classically known renin-angiotensin-aldosteron system and the antidiuretic hormone, special attention will be given to some of the newly described endocrine mechanisms. These newly described hormones are the so-called natriuretic hormone and the atrial natriuretic factors (ANF). Evidence for a natriuretic hormone goes back to the work by De Wardener in the early sixties (De Wardener and MacGregor, 1983). He found in the rat that during blood volume expansion, urinary sodium excretion increases. Plasma from a volume expanded rat causes natriuresis when infused in another rat. The natriuretic activity is caused by an ouabaine-like inhibitor of renal Na^+/K^+ ATPase. The inhibitor is possibly released by the central nervous system and originates from the anteroventral region of the third cerebral ventricle. Selective lesions in the preoptic hypothalamic periventricular region in the rat reduces the natriuresis after volume expansion by decreasing the hormone-like activity in plasma.

Evidence for the existence of natriuretic factors in the atrium which differ from the originally described natriuretic hormone, comes from the work of DeBold. In 1981, he and his co-workers demonstrated that the administration of atrial homogenates to rats results in a rapid massive, and short-lasting diuresis and natriuresis (DeBold et al, 1981). Later experiments led to the isolation and identification of these artrial natriuretic substances (Thibault et al, 1983). They are usually referred to as atriopeptins or atrial natriuretic factors (ANF). It soon became clear that atrial natriuretic factors are polypeptides and differ both chemically and biologically markedly from the natriuretic hormone (Maack et al, 1985). The atria contain considerable quantities of large molecular weight prohormone forms of ANF which are derived upon exocytosis. They are released upon atrial stretch (Palluk et al, 1985). Besides natriuresis and diuresis, it produces a concomitant phosphaturia, chloriduria, magnesuria, and a slight kaliuresis (Cantin and Genest, 1985). Furthermore, ANF increases the glomerular filtration rate (GFR) at high doses by increasing the glomerular capillary permeability. Recent microvascular studies of the kidney demonstrate a preglomerular dilation and postglomerular constriction in superficial cortical nephrons (Marin-Grez et al, 1986). ANF antagonizes vascular smooth muscle contraction induced by a variety of vaso-active agents in vitro and lowers blood pressure in the intact animal. The hypotensive effect of ANF, which has been confirmed in a variety of hypertensive models, has been attributed to the vasorelaxant effect observed in vitro, although conclusive evidence has not yet been reported. By itself ANF have no effect on the basal vascular muscular tone and vasorelaxation is independent of the presence of the endothelium.

Moreover, injection of ANF increases the urinary excretion of cyclic GMP, an important mediator of the relaxation of vascular smooth muscle (Ohlstein et al, 1985). Attempts to demonstrate the vasorelaxant activity in intact conscious animals (e.g. instrumented for measurement of regional blood flows) were negative (Lappe et al, 1985). Following continuous infusions of ANF, blood flow to the splanchnic viscera and kidneys decreased and vascular resistance increased. Recent evidence indicates that the hemodynamic responses are

greatly dependent on the mode of drug administration. The same peptide applied as bolus injection caused an increase in blood flow, indicating a reduced resistance (Lappe et al, 1986). The blood pressure lowering effect and its possible mechanism of action related to microvascular changes will be discussed in chapter 7 and 8.

The pathophysiological implications of ANF in hypertension are not clear yet. In hypertensive subjects and in animals during the established phase of spontaneous hypertension, plasma levels of ANF are markedly increased (Cantin and Genest, 1985; Takayanagi et al, 1986; Arendt et al, 1986) or normal (Yamaji et al, 1986), while atrial tissue levels of ANF are reported to be lower in hypertension. The plasma concentration changes are related to the progression of hypertension. The decrease of ANF levels in the left atrium seems to be due to an increased turn-over and in the SHR hypertension can occur despite increased levels of ANF.

1.3 The circulatory hemodynamics in the development of spontaneous hypertension in the rat

In the SHR, blood pressure rises with age (Smith et al, 1979; Prewitt et al, 1982; Evenwel et al, 1983). The blood pressure is above that of normotensive control Wistar or Wistar-Kyoto rats about 3-4 weeks after birth.

The increase in blood pressure in spontaneous hypertension may be the result of an increased cardiac output or elevation of peripheral resistance. An initiating factor in the development of essential hypertension seems to be the increased cardiac output. In young SHR (age 5-6 weeks), cardiac output is increased while peripheral resistance is still normal (Smith and Hutchins, 1979; Evenwel et al, 1983). Flow to the kidneys is decreased probably due to increased peripheral resistance as a consequence of an enhanced sympathetic activity initially triggered by afferent renal nerves (Kopp et al, 1987). Plasma volumes in young SHR are increased (Evenwel et al, 1983). An excess intravascular volume will lead to overperfusion of major vascular beds of the body. This induces a rise in tissue resistance to higher flow via autoregulatory mechanisms as originally formulated by Borst and

Borst-deGeus (1963) and Guyton et al (1971). High blood pressure becomes irreversible in the SHR by a genetic facilitation of cardiovascular structural adaption affecting the vessel wall or the microvascular architecture (Folkow, 1983). By that time, maintenance is independent of the sympathetic nervous system.

In parallel with the increase in cardiac output, intravascular volume becomes progressively contracted with a centripetal redistribution of intravascular volume towards the cardiopulmonary bed, most likely associated with a decreased compliance at the venous side (Folkow, 1983). The splanchnic vascular bed quickly increases resistance to the high blood pressure and/or flow (Evenwel et al, 1983). The last main vascular beds which increase resistance are the skin and skeletal muscle. The cerebrovascular bed shows an intermediate reaction. The microvascular features and their hemodynamic consequences will be discussed in the next sections.

1.4 Microcirculation and vascular resistance

No uniform definition of the microvascular bed can be given because among the several vascular beds the architectural design, branching order, configuration, vessel size and characteristics of blood flow are different. For an extensive review of microvascular beds, see Wiedeman (1981).

Usually the arterial vessel that marks the entrance into the microcirculation is called an arteriole and these vessels are classified by the method of Wiedeman (1963) into first, second, third and fourth order arterioles (A_1-A_4). An arteriole forms a functional unit with the surrounding tissue and has a diameter less than 100-125 μm. Arterioles terminate in precapillary arterioles which feed the capillary network. The precapillary arterioles control flow in capillaries. At the postcapillary side, venules converge into veins and have about 2-3 times the diameter of the arterioles and small arteries in the same vascular bed.

According to Poiseuille's law, the ratio of pressure drop to rate of flow is a function of all the factors that influence blood

flow (viscosity, length and radius) (Pappenheimer, 1984). This ratio has been adopted as an expression of vascular resistance by analogy with Ohm's law for electrical circuits. The resistance of a vascular bed is defined as the difference between the pressure at the inlet of the bed and the pressure at the outlet divided by the mean blood flow and peripheral resistance at frequency 0 Hz.

Several parameters may affect vascular resistance in hypertension. Since length of individual vessels can be regarded as constant in most beds and blood viscosity is constant under most conditions, it is evident from Poiseuille's law that resistance depends primarily on vessel radius. Resistance calculations of the microcirculatory bed are more complex. The pressure gradient within the network is a function of the diminishing diameter of the successive branching orders with the sharpest fall occurring in the region of the smallest arterioles. Recent studies, however indicate that a major fraction of the vascular resistance lies proximal to the microvascular bed. The resistance is modulated by feeding vessels located more upstream, supplying the microvascular bed (Segal and Duling, 1986; Meininger et al, 1987). These vessels have significant tone at rest, contribute to the overall vascular resistance and play a significant role in the regulation of tissue blood flow. Furthermore, the magnitude of the hydrostatic force driving the blood stream through the microvascular bed depends on the position of the microvascular bed relative to the main feed vessel (aorta).

Changes in vascular resistance are also related to the angioarchitecture of the microcirculation. Vessels may show equal diameters but an increase in vascular network resistance can still be provoked by a lowered number of parallel conductance channels (arteriolar segments of the same vessel order), i.e. rarefaction. Rarefaction is determined either functionally or structurally. Functional rarefaction refers to an increased number of arterioles temporary closed to flow in the resting condition while structural rarefaction refers to the lowered number of arterioles (1) functionally available following vasodilation or (2) anatomically present.

In addition, the involvement of a complex array of factors that include vessel length, diameter, branching in series versus parallel

deployement as well as rheological factors have to be considered (e.g. local hematocrit, leukocyte sticking, plasma skimming).

1.5 Vascular smooth muscle proliferation

The heart, arteries and arterioles are able to adapt their architecture by wall hypertrophy and or hyperplasia when sustained elevations of transmural pressure are at hand. Vascular structural changes in hypertension and related functional consequences have been discussed in recent review articles (Lee and Smeda, 1985; Lever, 1986; Schwartz et al, 1986). Blood vessels of all tissues are able to readily adapt their structure. This is not exclusively limited to arteries or arterioles. Also capacitance vessels show some structural adaptation that reduces venous compliance and contributes to blood volume centralization (Folkow, 1983). Morphological changes of blood vessels reducing the average inner radius of resistance vessels have important functional consequences leading to a steeper increase in vascular resistance following stimulation. The increased wall to lumen ratio results in a geometrically based vascular hyperreactivity (for a detailed discussion, see Folkow, 1983).

Vascular hypertrophy is involved in different types of hypertension (Lever, 1986). However, data on the role of vascular hypertrophy with regard to the aetiology of spontaneous hypertension remains scanty. An increased wall thickness may precede or develop as a consequence of the elevated blood pressure (Mulvany et al, 1978; Folkow, 1983; Lee, 1983, 1985). The available literature allows to conclude that hypertrophy of resistance vessels can be divided into three categories:

1. Pressure-dependent. In animals such as the giraffe, arteries in lower limb segments are ordinarily exposed to transmural pressures around 500 mm Hg due to combined hemodynamic and gravitational forces and their wall to lumen ratios are enormous compared with the cranial arteries (0.68-0.80 versus 0.15-0.20). The normalized blood flow for tissue weight is equal in skeletal muscle in the neck and in the feet (Pettersson et al, 1986). Other evidence for pressure-dependent hypertrophy is obtained from human studies.

Systemic veins in small children are largely similar in arms and legs. In adults, the raised hydrostatic pressure in the erect position affects venous wall to lumen ratio proportionally to the raised transmural pressure. Also veins used for arterial grafting very rapidly develop wall thickening (Folkow, 1983).

2. Increased wall thickness elicited by trophic effects. In earlier studies, it was proposed that sympathetic nerves exert a trophic effect on the developing vascular muscle. Denervation of the ear artery in normotensive rabbits reduced the weight and medial thickness of the vessel despite a normal perfusion pressure (Bevan, 1975). In SHR and stroke-prone SHR, sympathetic denervation prevented vascular hypertrophy of cerebral arteries and/or arterioles with a concomitantly altered autoregulation function (Hart et al, 1980; Sadoshima et al, 1986). It was concluded that sympathetic nerves may play an important protective role during chronic hypertension by contributing to cerebral vascular hypertrophy and maintain capillary pressure in the brain.

   The regulation of vascular growth is not fully understood but may be mediated by growth factors acting on specific receptors of the vessel wall. In essential hypertension, angiotensin II, insulin or insulin-like growth factor have been proposed as major endogenous stimulants (Lever et al, 1986).

3. Intrinsic changes linked to a genetic growth abnormality. The key question to be answered is whether structural vascular adaptations occur during the early pre-hypertensive stages. When all nervous and myogenic tone is removed from the smooth muscle the resistance remaining is only due to the structure or morphology of the vessel wall itself. Even if SHR are treated with pentolinium and papaverine before onset of high blood pressure structural adaptation still occurs by the 9th week of life as evidenced by an increased peripheral resistance following constant maximal vasodilation (Prewitt and Dowell, 1982). Also in other studies, long-term treatment with antihypertensive agents failed or only partly inhibited cardiovascular hypertrophy (Jespersen, 1986). The components of vascular hypertrophy (DNA replication, collagen, elastin) of the hypertensive type vary from one vessel to another and depend on the vas-

cular bed under study (Brayden et al, 1983). Vascular changes in the prehypertensive and early phase of spontaneous hypertension have been demonstrated for the splanchnic vascular bed (Lee, 1985), renal (Pang and Scott, 1981) and carotid vessels (Gray, 1982, 1984). An increased aortic DNA synthesis before blood pressure increased has been found in renal hypertensive rats (Loeb et al, 1986)

A feature described in the prehypertensive phase of spontaneous hypertension in large vessels is an enhanced DNA synthesis without karyokinesis or cytokinesis. This results in hypertrophic, polyploid cells that account for most of the increased mass of the hypertensive vessel (Schwartz et al, 1986). Small vessels on the other hand show a true hyperplasia that is, an increase in number of diploid cells. Recent evidence inidicates that only the largest arterioles and not precapillary arterioles show structural changes of the vessel wall (Miller et al, 1987). A higher DNA replication has also been found for cardiac and renal tissue of newborn SHR (Walter and Hamet, 1986).

Evidence of vessel wall hypertrophy as an obligatory step towards the development of hypertension is not conclusive yet. A predisposing genetic element has to be implied (Folkow, 1986).

1.6 Microvascular changes in spontaneous hypertension

The basic principles by which an increased microvascular resistance may be achieved have been mentioned in section 1.4. Because there is no general format of microvascular adaptation to chronic hypertension in the SHR, the main vascular beds and their reported vascular adaptation and relative importance to the increased resistance will be discussed separately. The majority of experiments have been performed on anesthetized animals with externalized tissues. A summary will be given of the animal models currently used with emphasis on the studies performed in the SHR.

1.6.1 Skeletal muscle

The microvasculature of skeletal muscle plays an important role in the development and maintenance of spontaneous hypertension (Evenwel et al, 1983). In the established phase of most forms of hypertension vascular resistance in skeletal muscle is elevated (Mueller, 1983) and the stiffness of resistance and capacitance vessels is increased. In addition, the minimum resistance measured after complete vasodilation is increased suggesting structural alterations of the vascular network (Mueller et al, 1983). The majority of experimental data have been obtained from the rat cremaster, gracilis and spinotrapezius muscles. These preparations are suitable because of a relatively easy surgical approach.

A 50% reduction of the number of perfused arterioles at rest in the cremaster muscle of 6-week old SHR as compared to 6 week old WKY has been reported by Hutchins and Darnell (1974). The reduction of the number of skeletal muscle arterioles was observed in a period when the blood pressure of the SHR is rising rapidly. These findings have been expanded in later studies to show that at 5 weeks of age not only the number of perfused arterioles is reduced, but also the total number of arterioles functionally available (Hutchins, 1977; Hutchins et al, 1982). However, once the spontaneous hypertension is established (12-30 weeks) the total number of cremaster arterioles is actually higher as compared to normotensive Wistar-Kyoto rats, whereas the number of patent arterioles remains below normotensive levels.

Chen et al (1981) investigated the contribution of the sympathetic nerves to the maintenance of a reduced number of perfused vessels. By using quantitative stereological techniques, they assessed whether the reduction was anatomical or functional. The capillary density was determined in addition. The results indicate that after cutting the hypogastric nerve, thus abolishing sympathetic tone, there was no increased neurogenic tone present as evidenced by enhanced arteriolar constriction in SHR as compared to WKY. In the vasodilated state no differences in maximum diameters could be detected. Therefore, structural changes of the vascular wall seem very unlikely. Chen et al (1981) concluded that a reduced number of arterioles is the only mechanism involved in the increase in vascular resistance in the SHR

cremaster muscle. The reduced capillary density should prevent an increased filtration of fluid into the interstitium, thus preventing edema formation. Arteriolar diameters in the cremaster muscle showed no difference or even a tendency to be larger in SHR than in WKY at an age of 5-6 weeks (Hutchins and Darnell, 1974; Dusseau et al, 1979; Chen et al, 1981). Only one single report shows an enhanced vasodilating capacity in the cremaster muscle (Roy and Mayrovitz, 1982). In the gracilis muscle (Prewitt et al, 1982), enhanced levels of active constriction were seen which were age-dependent. Interestingly, in the older SHR animals the maximum diameters increased but the actual vasodilating capacity increased as well so that overall average tone was approximately the same. Prewitt (1982) compared this phenomenon with a tree with half of its smallest branches pruned and the larger ones reduced in diameter. The mechanical significance is unknown yet.

The dynamic aspects and time course of microvascular changes during the development of spontaneous hypertension can be nicely illustrated by the results of a study by Prewitt et al (1982). Similar to the findings in the cremaster muscle, capillary density was decreased in the control situation as well as in the vasodilated state in the gracilis muscle at the age of 6-8 weeks (Prewitt et al, 1982). No evidence was obtained for arteriolar rarefaction at the age of 6-8 weeks. By 12-14 weeks, arteriolar density was reduced in the innervated condition but not after vasodilation. The vessels were present in SHR but were not available to flow. By 16-18 weeks, arteriolar density was reduced significantly in SHR even after vasodilation with sodium nitroprusside. So, the development of rarefaction of arterioles proceeds from a state of no change at 6-8 weeks of age, a state of functional rarefaction at 12-14 weeks, and finally at 16-18 weeks, a state of functional and anatomical rarefaction. Capillary density was also reduced at 16-18 weeks but not to the same extent as arteriolar density.

Skalak and Schmid-Schönbein (1986) found a reduced capillary mesh-work density in the spinotrapezius muscle of adult SHR. Gray (1984) determined whether there are morphological alterations in skeletal muscle capillaries of the spinotrapezius muscle as evidenced by changes in density, length, and diameter during early and late

stages of the developmental phase of spontaneous hypertension in the rat. In neonatal (19 days old) and young adult (9-10 weeks old) rats of the SHR and WKY strains, the presence of capillaries was assessed by histological and intravital microscopic techniques. In contrast to the findings in experimental animals and human hypertensives, diameters and densities are slightly increased.

The arteriolar network of the spinotrapezius muscle was studied in detail by Engelson et al (1985). The topology of the vascular arrangement of the spinotrapezius muscle was investigated by means of carbon filling, thus allowing only information with regard to the vascular anatomy. Studies were performed on 16-20 week old WKY and SHR. A new branching scheme applied to the numerous arcades was introduced which accounted for all arterioles in this muscle. Analysis revealed that the length per unit volume of the SHR arcade arterioles increased and furthermore showed the presence of almost twice as many transverse trees per unit tissue volume in the SHR as compared to WKY rats. Transverse arterioles in the SHRs are shorter and show a trend towards fewer capillary endings. Smaller arteriolar diameters were only observed for arcade arterioles.

Zweifach et al (1981) found that microvascular pressures were already elevated in the young hypertensive rat in the spinotrapezius muscle, with a much sharper decline in micropressure across the arteriolar branchings of the SHR. In the cremaster muscle, similar data were obtained (Bohlen et al, 1977). In the young hypertensive rat, at the age of 7-8 weeks, micropressures were 30-35% higher than those measured in normotensive rats. The distribution of pressure through comparable segments of the microcirculation of both strains was essentially identical.

In contrast to genetic hypertension, arteriolar diameters are reduced in desoxycorticosterone acetate (DOCA) salt hypertension, and in the one-kidney, one-clip renovascular hypertensive and two-kidney, one-clip rats (Meiniger et al, 1981, 1984; Prewitt et al, 1984; Joshua et al, 1984). It appeared that in renovascular hypertension an increased arteriolar dilating capacity diminishes as structural mechanisms supersede the maintenance of peripheral resistances. By 8-10 weeks after clipping, the difference in vasoconstriction between

hypertensive rats and controls is no longer statistically significant (Prewitt et al, 1984). The fact that rarefaction occurred indicates that it is not a genetic trait of rats destined to become hypertensive but a mechanism of possible regulatory importance that can be activated in a genetically normal rat.

1.6.2 The splanchnic vascular bed

The splanchnic circulation receives a large portion of the cardiac output, contains a substantial blood volume, and provides a major share of the total body lymph flow and transvascular protein flux. Therefore, hypertension-induced changes in the vasculature of this region can play an important role in systemic hemodynamics (Nijhof et al, 1983). Flow distribution data comparing SHR to normotensive control rats indicate that in SHR splanchnic vascular resistance is elevated in proportion to the increase in arterial pressure (Nishiyama et al, 1976; Tobia et al, 1974). The status of splanchnic and intestinal (macro)circulation in several forms of hypertension have been discussed by Overbeck (1984).

It should be kept in mind that the splanchnic vascular bed is rather heterogeneous with respect to different physiological control mechanisms. The spatial organization and fine structure is adapted for the transport function of the alimentary tract (Lundgren, 1984). The knowledge about microvascular changes of the splanchnic bed in spontaneous hypertension is scanty and predominantly restricted to the tissues that are easily transilluminated.

Microvascular changes during the development of spontaneous hypertension were investigated in the intestine of 4-5 week, and 18-21 week old SHR and WKY. Bohlen (1983) selected and analyzed the various modes of vascular changes and their relative importance to the increased intestinal vascular resistance during hypertension in SHR. Observations were performed on a loop of jejunum in pentobarbital-anesthetized rats. The intestinal vascular characteristics of young hypertensive rats exhibited no structural changes of the vascular design. By 4-5 weeks, arteriolar dimensions did not differ between both strains. In mature SHR a permanent loss of about 30-35% of the second order arterioles and some loss of perfused third order and

smaller arterioles were the only evidence of rarefaction in the intestinal vasculature. Approximately 6% of the total number of third order arterioles were not available for flow and there was no permanent loss of small arterioles as was the case for similar vessels in skeletal muscle. The primary physical cause of increased vascular resistance in the small intestine was vasoconstriction of the largest and smallest arterioles. In the same study, vessel wall hypertrophy was present in the A3 vessels. The latter had smaller diameters in the passive state.

Furthermore, the effects of systemic arterial hypertension on intestinal microvascular pressures were assessed (Bohlen, 1983). It was of interest that despite elevated arteriolar pressures, the intestine is essentially spared from an increase of capillary pressures during hypertension. The ratio of microvascular pressures to mean arterial pressure was elevated in the larger arterioles but disproportionally lower in the terminal portion of the vascular bed. No micropressure data were presented for young rats. In summary, in the jejunum the largests resistance increase occurred in the smallest arterioles.

Structural differences in the mesentery microcirculation between WKY and SHR were documented by Henrich et al (1978) in mature rats (age approximately 20 weeks). No data were collected in young rats. A quantitative comparison was given of the entire microvascular network. The number of pre- and postcapillary vessels was found to be significantly reduced in SHR as compared to WKY. The capillaries were subdivided into arteriolar and venular as well as mid-capillaries the number of which was reduced in SHR to nearly one/third. The vascular network was not analyzed during vasodilation and no attempts were made to differentiate between functional and structural rarefaction. Precapillary arterioles had on the average increased diameters and the total vessel length of successive branching order vessels was evidently diminished. Wall thickening was found only for the largest arterioles in diameter. Structural changes at the venular side were apparent with an equally pronounced decrease in density of the smallest venules in diameter. Microvascular pressures were measured in a separate study (Hertel and Henrich, 1982). In the mature SHR micro-

pressures were elevated to nearly the same percentage as the arterial pressure for all types of arterioles.

1.6.3 Cutaneous vascular bed

Haack et al (1980) compared the cutaneous microvessels from 5-10 week old SHR and WKY rats. A small sample of abdominal skin was extended and fixed. Following preparation the tissue was photographed and subsequently analyzed by measuring diameters and counting the number of arterioles and venules. Structural, i.e. anatomical, differences with regard to the arteriolar density were observed for the fourth order arterioles in SHR which was lowered by 30%. The SHR had 28.5% fewer fourth order veins compared to WKY.

Mean internal diameters of the distal portion of the vascular bed were larger as well in SHR as compared to WKY. The mean branching angles of both arterial and venous vessels in the tissue from SHR were greater than the respective angles measured in normotensive animals, but the difference did not reach statistical significance.

1.6.4 Cerebral microvascular changes

A third major vascular bed is the cerebral circulation. Mechanisms to protect the exchange vasculature from elevated microvascular pressures and potential overperfusion is especially important in the brain, where overperfusion can lead to edema and encephalopathy. Some form of protection does exist in the SHR since it is well established that in SHR cerebral blood flow is maintained at normal or near normal levels and cerebral vascular resistance is increased (Evenwel et al, 1983). Very little data exist about microvascular changes of the cerebral microcirculation. Most observations were made in the microcirculation of the cerebral cortex of adult spontaneously hypertensive and normotensive rats. Harper and Bohlen (1984) were not able to demonstrate either functional or structural rarefaction in the adult, 18-21 week old, SHR as compared to WKY. Arteriolar density for several vessel categories was identical. Constriction of the larger arterioles was the major cause for the increased resistance of this part of the circulation, whereas arterioles with diameters less than 25 µm were not affected. In the vasodilated state, in the large arterioles a

greater wall thickness could be observed in SHR, indicating the presence of structural changes of the vessel wall in SHR. Furthermore, arterioles had a decreased diameter following maximal dilation. Within the microvascular bed, micropressures were elevated at all levels and in SHR the greatest pressure drop advanced towards the capillary bed in the smallest arterioles (A3 and A4).

The results of Harper and Bohlen (1984) are at variance with a later report of Sokolova et al (1985). Arteriolar and capillary networks in the brain of hypertensive and normotensive rats were analyzed in that study. Vessels were perfused with a 4% solution of gelatin in India ink with subsequent histological analysis. In a group of 3-month old rats with spontaneous hypertension, the number of third, fourth and fifth order branches was lower and their diameter smaller in hypertensive animals as compared to controls. Capillary density was studied rather extensively. In the same age group the intracerebral vessels were investigated. Histological examinations showed that the capillary network, as judged by a decreased length in the reticular nuclei of the medulla oblongata and the pons,was considerably smaller in the hypertensive animals.

Furthermore, Sokolova et al (1985) made an interesting observation of a rapid appearance of anatomical rarefaction in developing renal hypertension. A rise of pressure and decrease in the number of vessels are already apparent 24 hr in rats with a single ligated kidney. These data do implicate that structural changes of the vascular bed can be involved in a very early phase of hypertension.

1.6.5 Renal microvasculature

Although the kidneys play a crucial role in the development of spontaneous hypertension, surprisingly no systematic analysis has been made thus far regarding renal microvascular changes during the development of spontaneous hypertension using intravital microscopic techniques.

1.7 Synopsis

The multifactorial character of spontaneous hypertension in rats or essential hypertension in humans as a primary disease of the vascular bed has led investigators to somewhat different approaches concerning the genesis of hypertension. At a systemic level different factors and neurohumoral mechanisms can influence blood pressure and much emphasis has been put on central hemodynamic mechanisms being key ingredients of the progressive elevation of blood pressure with a concomitant rise in vascular resistance (Peart, 1983; Evenwel et al, 1983).

Studies focussed on the central as well as the regional hemodynamics found an increased peripheral resistance with an equally distributed cardiac output in the phase of stable hypertension. The time profile for the appearance of the increased resistance depends upon the particular vascular beds (Evenwel et al, 1983).

In the SHR at the age of 5 weeks the kidney is the first to increase its resistance closely followed by the splanchnic bed. In the adult rats (10-12 weeks) the skeletal muscle and skin have increased their resistance and blood pressure plateau to a steady-state elevated level. Interestingly, in the SHR the resistance during aging is in a way relative. In fact, during aging WKY decrease their total peripheral resistance suggesting that the SHR has a hampered vascular outgrowth.

The increase in vascular resistance should be reflected by microvascular changes. Since a steep pressure drop at a given organ will occur within the microvasculature the importance of microvascular research in order to disclose the aetiology of spontaneous hypertension is obvious.

The use of intravital microscopy has allowed investigators to evaluate the status of the microcirculation in selected tissues, primarily of parenchymal tissues (Wiedeman et al, 1981). It is difficult to fit all data into one perfectly fitting scheme because differences with regard to methodology, animal species, strains and age, and anesthesia, have to be considered.

In-vivo observation of almost every major vascular bed has been

used to determine whether and where in the vasculature there is an unusual amount of active or passive (i.e. increased wall to lumen ratio reducing maximal lumen size) vasoconstriction. Most studies fail to demonstrate an enhanced vasodilating capacity during the developmental phase of spontaneous hypertension (Zweifach, 1981). Another possibility is that the increased resistance is determined by small, more upstream located arteries which are usually not observed in standard microvascular preparations (Segal and Duling, 1986; Meininger et al, 1987). Lumen diameters tend to be increased in larger arterioles of SHR, whereas they tend to be smaller in the end arterioles (Zweifach et al, 1981). Changes in vessel morphology and a decreased distensibility of larger arterioles or arteries may occur, but seem to play a minor role in the aetiology (Hutchins et al, 1982; Miller et al, 1987). Vessel wall hypertrophy and constriction are usually interpreted to be indicative of long existing and profound hypertension. In SHR arteriolar responsiveness to vaso-active stimuli is enhanced as well by that time.

A second mechanism by which vascular resistance may be increased is rarefaction (Hutchins and Darnell, 1974; Chen, 1981; Hutchins, 1982; Prewitt et al, 1982). The number of arterioles available to the circulation is reduced under resting conditions in SHR. The closure may be temporary or permanent (Prewitt et al, 1982). The primary underlying mechanism of anatomical arteriolar rarefaction is unknown. Although it may be attributed to a genetic predisposition leading to rarefaction and retardation in vascular growth, arteriolar and capillary rarefaction will also develop in genetically normal rats with an increased blood pressure (Prewitt et al, 1984; Sokolova et al, 1985; Meininger et al, 1986). It is yet controversial whether a functional rarefaction precedes a structural change although it is conceivable that long-term closure as a consequence of an increased arteriolar responsiveness to vaso-active stimuli will lead to irreversible closure.

In some tissues network reconstruction shows a higher density of arcade type of arterioles although not specific for all tissues (Engelson et al, 1985). Furthermore, it is difficult to correlate the occurrence of rarefaction in different vascular beds and the develop-

ment of hypertension in the SHR. Even within the same type of tissue different microvascular derangements are manifest at different ages.

Although the reduction in systemic blood pressure along the vascular tree follows essentially the same profile in both SHR and WKY, micropressures are slightly increased in the 20-30 μm arterioles and are near normal levels in capillaries and venules (Zweifach and Lidowski, 1984). The fall in arteriolar blood pressure in all beds studied in the SHR is 2 to 3 times greater than normal (Bohlen 1977, 1983; Harper and Bohlen, 1984; Zweifach 1984).

Although a higher than normal resistance in the small arterioles may help to minimize increased capillary perfusion and filtration during hypertension, segmental resistance along the vascular bed may be increased more upstream and therefore not be confined to the microcirculation per se. Assuming that in adult SHR total peripheral resistance is elevated by 50%, small arterioles are responsible for 10-15% of the increase seen in SHR (Bohlen, 1986). For the remaining 35-40% increase in resistance small arteries and large arterioles must be considered.

In view of the existence of mechanisms subserving both systemic and local needs, it would seem plausible to assume that no singular factor can explain either the initiating or sustaining feature of spontaneous hypertension based on microcirculatory evidence (Bohlen, 1986).

In summary, all data lead us to propose the following hypothesis with regard to vascular changes occurring in hypertension. Within the most distal part of the circulation density of capillaries and the smallest arterioles will diminish due to an unknown factor or to a mechanism preventing normal maturation of the vascular bed. This will result in a modest increase of arterial blood pressure. The increased blood pressure will affect the genetically predisposed larger vessels as well. Larger vessels are not able to undergo rarefaction, but change their functional characteristics by an increased wall thickness or hyperresponsiveness for pressor stimuli. If larger arterioles and small arteries become affected blood pressure may rise even more steeply and ultimately stabilize at a fixed level.

1.8 The present thesis

Many theories have been put forward to explain the progressive rise in blood pressure with the concomitant increase in peripheral resistance in spontaneous hypertension. Factors such as renal or neurohumoral dysfunction or increased responsiveness of the vasculature can increase the peripheral resistance and have received a considerable amount of attention in the past (see general introduction; Zweifach et al, 1981).

It has been hypothesized that structural changes lead to a fixed hyperresponsiveness of the arterial vascular system and cause sustained hypertension (Folkow, 1983; Lee and Smeda, 1985; Lever, 1986). Furthermore, the design of the microvascular bed and its related physiological function may be changed and contribute to the development of spontaneous hypertension (Hutchins and Darnell, 1974; Henrich et al, 1978; Hutchins, 1979; Chen et al, 1981; Prewitt et al, 1982; Bohlen, 1983; Harper and Bohlen, 1984).

A large amount of research has been focussed at these mechanisms through the use of whole animal studies, organ studies and in-vitro analysis of isolated blood vessels.

Peripheral control of blood pressure and control of the microcirculation occurs at the arteriolar level of the microcirculation. Most methods currently employed to assess vascular changes only reflect these changes and do not measure them directly. It is therefore a logic consequence to attempt to study them by direct means. By using intravital microscopy, relevant parameters of microvascular hemodynamics can be measured in vivo and on-line. In this way, microvascular disturbances can directly be related to the pathophysiological processes involved in spontaneous hypertension.

The aim of the present thesis was to study some aspects of spontaneous hypertension related to vascular, neurogenic and humoral microcirculatory events. The first aim of this study was to investigate the microcirculatory changes during the early developmental phase of spontaneous hypertension in a selected tissue in order to shed more light on the cause and effect relationship. In chapter 3 such a study is described for the cremaster vascular bed. In particular, the func-

tional aspects with regard to blood flow regulation had not been studied in detail in previous studies. Therefore, special attention to this aspect is given in chapter 3. In chapter 4, an attempt was made to elucidate microvascular changes involved in the maintenance phase of spontaneous hypertension in adult rats. This study was done in conscious animals not exposed to anesthetics. In chapter 5, we investigated whether sympathetic stimulation will result in an enhanced arteriolar tone. Sympathetic adrenergic stimuli were applied in different ways and the stimulus effect relationship was studied and correlated to corresponding microvascular changes. Studies on drug effects in the microcirculation always suffer from covariance caused by the anesthetic. We therefore describe in chapter 6 a study on the influence of several anesthetics frequently used on the control of vascular resistance in different beds. The influence of atrial peptides was studied as a hormonal mediator of cardiovascular homeostasis and blood pressure regulation. Its blood pressure lowering mechanism is only partially disclosed and its role in the development of spontaneous hypertension unknown. The microcirculatory studies on ANF described in chapters 7 and 8 aimed at a further analysis of the vascular effects of these hormones.

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2. MATERIALS AND METHODS

2.1 Animals

In all studies, experiments were performed on male rats. Depending on the experimental protocol, rats derived from different strains were used. Spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) were obtained from the breeding colony of the Central Animal Facilities of our university. Wistar rats were purchased from the Centraal Proefdieren Bedrijf, TNO (Zeist, The Netherlands). In one study presented in chapter 4, SHR and WKY were purchased from another supplier (Charles River Lab Inc., Portage, Mi, USA) but they were originally derived from the same strain. These rats were shipped in pathogen-free boxes and were allowed to recover from any trauma during shipment for 1-2 weeks.

The SHR strain was intentionally developed as a genetic animal model of human essential hypertension by Okamoto and Aoki in the early sixties (Udenfriend et al, 1976). Although there are other animal models to study hypertension, the SHR was chosen because of the wealth of information obtained with this model, and furthermore, it is considered to be representative of essential hypertension in man (Trippodo and Frohlich, 1981). For comparative purposes the parent WKY strain served as an adequate control.

Before and between the experiments, when using conscious animals, rats had free access to standard lab food and tap water. Regular food was obtained from Hope farms (Woerden, The Netherlands).

2.2 Anesthesia

A variety of anesthetics or combinations of anesthetics was used depending on the experimental design. Implantation of catheters in the femoral artery and vein was usually carried out under light anesthesia by ether inhalation or by an intramuscular injection of a combination of Rompun (xylazin, HCl 2% solution; Bayer 0.6 ml/100 g body weight) and Vetalar (100 mg/ml ketamine hydrochloride, Park Davis: 0.3 ml/kg).

The latter combination was also used for the implantation of stimulation electrodes in the posterior hypothalamus.

For implantation of Doppler flow probes and of the dorsal microcirculatory chamber, the anesthetic sodium pentobarbital (Narcovet^{R}, Apharma, Arnhem, The Netherlands) was used. Sodium pentobarbital was administered intraperitoneally (i.p.) (60 mg/kg).

When using the mesentery preparation, rats were anesthetized by a slow infusion of sodium pentobarbital (30 mg/kg) through a PE-10 catheter which had been inserted into the left femoral vein at least 3 days prior to the experiment. In some experiments, the tail vein was acutely cannulated as alternative. Following induction of anesthesia, a steady-state anesthetic depth was achieved by a slow continuous infusion of a pentobarbital solution (60 mg/ml) at a rate of 26 mg/kg/hr using a Harvard infusion pump (Millis, Mass, USA; model 975 A). With the infusion, the rat received an additional volume load of 2.5 µl/min.

The rat cremaster preparation was carried out following an intraperitoneal injection (0.6 ml/100 g body weight) of a chloralose urethane mixture (1-13.3%) dissolved in physiological saline (0.9% NaCl). The solution had to be warmed (50-60^{o}C) to improve solubility and was cooled to a temperature of approximately 40^{o}C before it was administered. Small doses of supplemental anesthesia were necessary after 2-3 hours. Urethane was purchased from Fluka AG (Buchs, Switzerland) and alpha-chloralose from Sigma (St. Louis, Mo, USA).

Following induction of anesthesia, body temperature was kept constant (37^{o}C) by placing the rat on a water-filled heating pad (Aquamatic K-module, Cincinnati, OH, USA). For acute experiments, a tracheotomy was performed by cutting the trachea between two rings of cartilage and inserting a 2-cm long section of PE-200 tubing to a depth of 1 cm.

2.3 Blood pressure measurements

In most experiments, blood pressure was measured through a catheter in the abdominal aorta. In the experiments in chapter 3 using

the cremaster preparation, the carotid artery was cannulated to exclude any induced mechanical obstruction or flow redistribution in the hindquarters.

The technique for chronic measurement of blood pressure has been extensively described elsewhere (Smits, 1980). In short, a catheter was constructed from a 9-cm piece of PE-10 tubing which was heat-sealed to a 12-cm piece of PE-50 tubing. On the other end of the PE-50 tubing, a 1-cm piece of PE-100 tubing was heat-sealed. A 3-cm piece of vinyl tubing (Serva TT 63) was slipped over it. Vinyl tubing permits frequent clamping without damaging it. For implantation of the catheter described above, it was necessary to make a small incision in the left groin using aseptic techniques. The femoral artery was freed from connective tissue and following clamping, a small hole was cut in it with ophthalmologic scissors and the PE-10 catheter was inserted. It was advanced for 4 cm into the artery so that its tip was 1 cm above the bifurcation and below the renal arteries. The catheter was secured to the artery and to the underlying muscles with silk sutures. When in the experiments conscious rats were required, the catheter was guided subcutaneously (s.c.) to the neck where it was exteriorized. The catheter was then filled with heparinized saline (250 IU/ml) and closed with a metal obturator.

For chronic microvascular studies, using the dorsal microcirculatory chamber, a different approach was chosen, because it is difficult to guide the catheter subcutaneously to the neck in the presence of the chamber. The tail was sterilized and a small skin incision was made at the ventral side. The tail artery was carefully dissected and care was taken not to damage the concomitant veins. The PE-10 tubing (length 8-9 cm) which had been previously sealed to PE-50 tubing (length 45 cm) was inserted until its tip reached the abdominal aorta just above the bifurcation. The catheter was fixed to the tail by using 2 silk sutures. The wound was closed and covered with Steristrips. A metal spring-stock (Instech Laboratories, Horsham, PA, USA) which served to protect the catheter from biting was secured to the tail with 2 metal stitches (Hutchins et al, 1986). The position of the catheter was verified post-mortem.

The carotid artery was only cannulated for acute measurement of

blood pressure. A catheter was constructed from a 4-cm piece of PE-10 tubing and heat-sealed to a PE-50 tubing (length 30-40 cm). A ventral incision was made in the neck and the left carotid artery was dissected free from surrounding tissue. The PE-10 catheter was inserted into it through a small hole which was cut in the artery with ophthalmologic scissors. The catheter was secured to the vessel with silk sutures and exteriorized. The same type of catheter (PE 10) was used for cannulation of the jugular vein for intravenous injections of FITC-BSA after instalment of the catheter. The wound was closed.

A low-volume displacement transducer (CP 01; CTC, Inglewood, Ca, USA) was used to measure blood pressure. To ensure patency of the catheter during the experiments, a continuous infusion of a Ringer solution was given (1 ml/hr) using an infusion pump (Precidor Infors AG, Basel, Switzerland) and an intra-flow device (CFS Intraflo II CF S2-03F; Sorenson Research, Salt Lake City, Utah, USA). All signals were recorded on a Grass Model 7D polygraph (Grass Instruments, Quincy, Mass, USA) or on a Schwarzer recorder model RS 348 (Schwarzer GmbH, München, GFR).

Mean arterial pressure (MAP) was obtained by low-pass filtering of the pulsatile blood pressure signal. Heart rate (HR) was determined from the pulsatile blood pressure signal by a biotachometer.

Central hemodynamic parameters (MAP, HR) during the experiments described in chapter 4 were measured using a 150 PC flow thru pressure sensor (Micro Switch, Honeywell, Freeport, Il, USA) which permits a simultaneous infusion of heparinized saline (2 ml/day, 200 IU/ml). Hemodynamic parameters in these experiments were continuously monitored for 24 hr a day (MAP, HR), using a Sanyo-MBL-550 computer and displayed on a Mitsubishi monitor. Mean values of each parameter were printed every hour and used for data analysis.

2.4 Administration of drugs

Drugs were administered by different routes. If drugs were applied systemically, the right femoral vein was used. To this purpose, bolus injections with drugs in different concentrations in a volume of

50 ul (Hamilton Bonaduz AG, Bonaduz, Swiss) or continuous infusions with different infusion rates were performed. Between the administration of bolus injections 10 min were allowed for recovery.

Using the mesentery preparation, drugs were also applied locally via the superior mesenteric artery or topically with the drug dissolved in Tyrode's solution.

2.4.1 Cannulation of the right femoral vein

The right groin of the rat was shaven and a small incision was made. The femoral vein was freed from connective tissue by gentle dissection. A small hole was cut in it and the PE-10 catheter was inserted until its tip reached the inferior caval vein. The catheter was fixed to the muscle and the wound closed.

2.4.2 Cannulation of a small side-branch of the superior mesenteric artery

Local administration of vaso-active drugs has been successfully performed in earlier studies (Kleinjans et al, 1984; Smits and Thijssen, 1986). Therefore, in a subset of experiment using the mesentery preparation, vasopressor substances were injected into the feeding artery.

A small upper mid-abdominal incision was made, and the intestines were carefully spread to the left side of the rat and wrapped in moistened gauzes. Care was taken not to touch the coecum or the distal ileum containing the mesentery. The superior mesenteric artery could easily be recognized by its splanchnic nervous plexus and lymph vessels which run in parallel. One of the first small side-branches of the superior mesenteric artery was dissected free of fat tissue under a stereo microscope (Wild M7S; Heerbrugg, Switzerland), using glass rods to minimize trauma. A pulled PE-10 catheter was retrogradely inserted into the vessel without obstructing mesenteric blood flow in the main artery. The catheter was guided outside and fixed to the abdominal wall with one 2-0 silk suture. The wound was closed. The method is basically the same as described for cannulation of the suprarenal artery (Smits et al, 1983). To verify the location, the catheter was flushed with 200 µl indian ink or a methylene blue solution after the experiment.

2.4.3 Topical administration of drugs

In case drugs were applied topically on the mesentery preparation, a catheter (PE 50) was firmly attached with tape to the salt water immersion objective. The regular superfusion with Tyrode's solution was stopped and within 3-5 min a maximal volume of 40-50 ml Tyrode's solution with the drug dissolved in it was directly superfused on the preparation, to obtain the correct drug concentration. Subsequently the mesentery was superfused at the regular superfusion rate. Following completion of the protocol, superfusion with regular Tyrode's was started, first at a high rate to wash away the drug, followed by the regular superfusion rate. In these experiments 10 min were waited to regain base-line values.

2.5 Regional blood flow measurement

Doppler flowmetry was chosen as a technique to assess regional blood flow because dynamic changes can be quantitated. Furthermore, the minute size of the Doppler flow probes, as compared to the classical bulky electromagnetic probes, enabled us to study splanchnic blood flow and to correlate this with microvascular alterations in the same preparation.

2.5.1 Doppler flowmetry

A 545 C-3 directional pulsed Doppler flowmeter (Bioengineering Resource Facility, University of Iowa, Iowa City, IA, USA) with miniaturized Doppler flow probes, positioned around the superior mesenteric artery, the renal artery and abdominal aorta, was used to measure regional blood flow. Following an upper midabdominal incision the origin of the superior mesenteric artery was dissected free of surrounding tissue. The intestines were spread to the left side of the rat and were kept moistened with gauzes and wrapped in aluminum. The silastic cuff of the probe (inner diameter 0.9 mm) was fixed around the superior mesenteric artery. In some experiments, Doppler flow probes were also placed around the left renal artery (0.6-0.8 mm) and the abdominal aorta (1.2 mm) distal to the iliolumbar arteries. Flow

through the abdominal aorta consists mainly of skeletal muscle blood flow and will be referred to as hindquarter flow. The contact of the probe with the vessel wall was improved by applying ultrasound coupling gel (ultrasound coupling gel, Pie Data Medical, Maastricht, The Netherlands). The wires were fixed at the inside of the abdominal muscles to prevent traction on the probe and were guided outside. Finally, the abdomen was closed with silk sutures. In case conscious rats were needed the wires were guided subcutaneously to a connector which was fixed to the skull with jeweller's screws and dental cement. These rats were allowed to recover from surgery for at least 3 days before implantation of intra-arterial and intravenous catheters.

The method of measuring regional blood flow has been extensively described elsewhere (Haywood et al, 1981; Smits and Struyker Boudier, 1984). The Doppler frequency shift is a function of the velocity of the red blood cells and therefore provides a relative parameter of the blood flow. Relative changes in total organ vascular resistance were calculated according to the following formula:

$$\left[\frac{1 + \frac{\Delta MAP}{MAP}}{1 + \frac{\Delta flow}{flow}} - 1 \right] \times 100\%$$

2.6 Posterior hypothalamus stimulation

The influence of increased sympathetic tone on blood flow in mesenteric arterioles was investigated by electric stimulation of the posterior hypothalamus. Stimulation of this nucleus results in a general increased tone to all innervated organs (Ninomiya et al, 1970). To minimize the secondary influence of circulating catecholamines released by the adrenals upon hypothalamic stimulation, resection of both adrenals was carried out as well.

At least three days before the experiments, animals were anesthetized and mounted in a stereotaxic frame (David Kopf, Tujunga, Ca, USA). A stainless steel bipolar electrode (MS/-303/2, Plastic Products

Company Roanoke, VA, USA) was implanted at A: 0.3 mm, L: 0.6 mm, H: -2.4 mm according to a standard atlas (König and Klippel, 1963). The electrode was fixed to the skull with dental cement and the skin was closed around it. The bilateral adrenalectomy was performed on the experimental day. To this end, two small flank incisions were made at the right and left side of the rat prior to the mesentery preparation. The adrenals were removed retroperitoneally and the wound was closed.

The bipolar electrode was connected to the stimulator (Grass, Quincy, Mass, USA) when the rat was mounted on the animal stage. Stimulations were performed with alternating voltage stimuli (2 V; pulse width of 0.5 ms). Frequencies were set at 8, 16, 32, and 64 Hz, respectively. The output of the stimulator was connected to an oscilloscope (Philips PM 3540) for visual control of the stimulation parameters. Stimulation was continued until maximal effects were reached, generally for 20-50 s. Between stimulations, 10 minutes were allowed for recovery. At the end of the experiment, the animals were sacrificed and perfused with formaldehyde (10%) in saline (0.9% NaCl). The brains were removed and stored in 4% formaldehyde for 4-7 days. The position of the electrode was verified by comparison of consecutive slices of the brain to the atlas of König and Klippel. Only experiments from animals with correct electrode location were used.

2.7 Anesthetized animal preparations

2.7.1 Mesentery preparation

The mesentery of rats is widely used for intravital microscopy of the microcirculation and is regarded as representative of the splanchnic vascular bed.

Following induction of anesthesia (section 2.2), the rat was shaved at its right flank. Loose hair was removed. With a scalpel blade a small right flank incision (approximately 2 cm) was made through the skin and underlying abdominal muscles. Small bleeding spots were coagulated immediately.

The mesentery was exteriorized through the flank incision. The coecum and a short segment of the distal ileum were brought outside

and the mesentery was carefully spread with wet cotton wool sticks over a siliconized glass plate mounted on an electrically heated (37-38°C) microscope stage. Care was taken not to touch or stretch the mesentery to avoid amine and prostaglandin induced vasodilation and disappearance of vasodilating capacity of arterioles (Henrich et al, 1978). The preparation was immediately superfused with Tyrode's solution to prevent dehydration. The intestines were covered with moistened gauzes. The Tyrode's solution had the following composition in mM: NaCl 130, KCl 5.6, $CaCl_2$ 2.2, $MgCl_2$ 1.7, $NaHCO_3$ 24, NaH_2PO_4 12, glucose 11, and saccharose 13. The pH was kept at 7.35-7.45 by bubbling the solution with a gas mixture of 5% CO_2 and 95% N_2 at a temperature of 36-37°C (Reneman et al, 1980). The superfusion rate was 6 ml/min.

The mesentery of the three most distally situated loops of the ileum was inspected at low magnification. Only preparations with minimal leukocyte sticking, minimal capillary stasis and minimal microbleedings were used. The integrity of the preparation can easily be assessed by the occurence of complete arteriolar closure following topical application of noradrenaline (10^{-6} M) or the absence of leakage of intravenously administered florescein isothiocyanate bovine serum albumin (FITC-BSA) (200 mg/kg). Although the mesentery is regarded as representative of the intestinal microcirculation, its physiological role remains unknown. It may be important for fat storage and mobilization, or contribute to the overall fluid balance of the peritoneal cavity, given the large surface area of the mesentery exposed to peritoneal fluid around the mesentery. The intestinal microcirculaton including the mesentery is a rather unique microvascular bed, because its venous drainage is via the portal vein into the liver, i.e. coupled in series, with the liver before flow returns to the heart. Hence, venous pressures should be higher as compared to other vascular beds (Gore and Bohlen, 1977). Often lymph vessels can be noted, which exhibit slow rhythmic contractile activity and propulsion of lymph. These vessels drain into the thoracic duct. As a rule, no vasomotion was present in all arterioles down to the precapillary vessels. Exceptionally, vasomotion could be observed in those arterioles which were directly situated between or close to fat cells

surrounding the main arteriole feeding the intestinal loop.

In young rats with body weights lower than 270 grams, usually no mesenteric microvessels were present. Therefore, only rats with a minimum weight of 275 grams were used for these experiments.

2.7.2 Intestinal preparation

The same experimental approach for externalizing the mesentery was used when the intestinal microcirculation of the distal ileum was studied. Larger vessels are located at the outer plexus of the ileum or muscularis externa and originate from the distal branches of the superior mesenteric artery close to the mesenteric border. From the mesenteric border, main feeding arterioles and draining venules run around the intestinal wall and end at the antimesenteric border. In contrast to the preparation technique described by Bohlen and Gore (1976), which includes dissection at the antimesenteric border of the jejunum to facilitate transillumination, the intestinal loop was left intact and no drug had to be used to control intestinal motility.

To exclude the influence from room oxygen often reported to cause vascular constriction or loss of autoregulatory responses (House and Johnson, 1986), the intestinal loop was covered by Saran Wrap^{R} (Dow Chemical Company, Indianapolis, Indiana, USA), a thin sheet of polyvinylidene chloride foil that has a low permeability for oxygen.

2.7.3 The cremaster preparation

Striated muscle plays a pivotal role in systemic hemodynamic and blood pressure regulation in hypertension. The cremaster muscle is composed of striated muscle and is widely used to study microvascular changes in hypertension due to its accessibility and transparency. This muscle can easily be transilluminated with minimal trauma.

The cremaster pouch is mainly composed of structural components of the external oblique and transversal abdominal muscles (Baez, 1973). The muscle composition as determined by histochemical techniques and expressed as the ratio of number of fibers per cross-sectional area is predominantly (60-80%) type IIb. This muscle can be regarded histochemically as an example of skeletal muscle but not functionally (Sarelius et al, 1983), because it is not involved in

locomotion. The muscle consists of two layers, each about four fibers thick. Sometimes, fibers run at different angles, varying between different preparations. The anteromedial part of the muscle has the highest transparency and is preferred to perform microcirculatory observations by most authors.

The cremaster muscle is directly supplied with blood by distal branches of the external spermatic artery. This artery branches off from the pudic-epigastric artery, the main feeding artery of which is the common iliac artery. The anatomic arrangement and variations in the origin of the feeding arteries of the rat cremaster microcirculation have been determined in detail by Meininger et al (1987). Due to its anatomical location, the in-situ temperature of the cremaster is reported to be a few degrees below body temperature (Hutchins and Darnell, 1974). The normal in situ temperature ranges between 34-35^{o}C.

Cremaster exposure and dissection procedures were adapted from Baez (1973). The anterior aspect of the scrotum was shaved and loose hair was carefully removed. With the rat in the supine position, the scrotum was moderately extended by means of an anchoring silk suture (2-0) through the hind pole. A longitudinal incision of skin and fascia was made in the midline over the ventral aspect of the scrotum. From the ventral surface the left testis was exposed and lifted from the scrotum and its length was measured. Tyrode's solution at room temperature was dripped on the preparation to prevent dehydration. The connective tissue was carefully stripped away with as little damage as possible to obtain optimal clarity of the preparation. The enveloping cremaster muscle was then unwrapped from the testis by making a ventral incision along the entire length of the muscle with a microcauter. This incision was made directly opposite the dorsally positioned primary arteriole and the neural connections. The testis and epididymal fat pad were gently pulled free. The meso-epididymis was cauterized and cut with ophthalmologic scissors. The epididymis and testis were advanced into the abdominal cavity. A small cotton ball fitting into the inguinal canal prevented extrusion of these organs in the course of the experiments and leakage of Tyrode's solution into the abdominal cavity. Seven silk sutures were attached at the periphery of the cremaster and the muscle was stretched in a drum-head

fashion in the open tissue bath. The length of the muscle was adjusted to its original length by applying an even tension to the cremaster. Tension applied in this manner is sufficient to provide good visualization and to maintain reproducible vascular tone and reactivity (Baez, 1973). The tissue bath (volume approximately 8 ml) was mounted on the microscope stage. The cremaster bath was continuously perfused at a rate of 6 ml/min with a temperature-controlled Tyrode's solution, saturated with 95% N_2 and 5% CO_2 (34.5°C). Between the animal and the optical port, high-vacuum grease (S.A. Dow Corning, Seneffe, Belgium) served to seal the open organ bath. Cremaster bath CO_2 and O_2 partial pressures were 40 mmHg and <30 mmHg, respectively. The muscle preparation was allowed to equilibrate 45-60 min prior to the start of the experiment to allow for restoration of vascular tone and vasomotion (Le Noble et al, 1986). Occasionally, however, preparations were discarded showing slowing down of capillary blood flow, excessive adherence of leukocytes to the venular walls or petechial bleedings.

Spontaneous, localized fasciculations of muscle fibers were effectively eliminated by applying topically a diluted muscle-relaxant solution (Curarin-Asta 1%, Bielefeld, Germany; 250 µl of a 1:10 diluted solution). This effect lasted 30-45 min. Muscle relaxants were found not to alter vascular reactivity in cremaster muscle (Baez, 1973; Faber et al, 1982). High concentrations, however, can result in systemic drug absorption and death possibly by respiratory paralysis.

2.8 Conscious animal preparation

A few (superficial) tissues in the rat allow for direct microscopic observations without surgical approach and the necessity of anesthetics. The microcirculatory chamber for conscious animals was first developed for the rabbit ear which mainly consists of newly developed tissue (scar tissue) (Sandison, 1924). The technique was modified for implantation in hamsters (Endrich et al, 1980) and rats (Papenfuss et al, 1979) allowing the study of preformed skin tissue. Recently, a new model of the dorsal microcirculatory chamber was designed which offers the possibility to study preformed muscular

tissue in conscious rats during approximately 2 months (Smith et al, 1985).

Before the implantation of the dorsal microcirculatory chamber, rats have to get used to handling by the investigator. A training protocol for animal restraining started at least 1-2 weeks prior to surgery. At the end of the training protocol, rats have to sit in the restrainer loosely restrained for a period of 90-120 min. As soon as rats had recovered from surgery the training protocol was continued.

2.8.1 The dorsal microcirculatory chamber (DMC)

The study presented in chapter 4 is based upon experiments with a two-sided muscle layer preparation as originally described by Smith et al (1985). Chambers were obtained from Carolina Medical Electronics, King, NC, USA (see fig. 2.1) and consisted of two halves. Each half, consisting of a support frame and inner ring, was made of thermoneutral polycarbonate and was injection-molded. Sharp edges were smoothened to prevent animal irritation and tissue damage following implantation. The inner ring was counter-sunk to accommodate the cover slip. Cover slips, no 1.5, were obtained from Labtek division (Naperville, IL, USA) and were sealed with epoxy adhesive. Side B of the DMC was fitted with stainless steel pins (0.61 mm in diameter). These pins matched sockets in side A and maintained a cover-glass to cover-glass distance (between A and B) of 800-1000 μm preventing compression of the interposed muscular tissue. This distance was based upon a two-sided muscle layer preparation. In a follow-up study considerable increase in optical clarity was achieved by removing the (muscle) front layer during surgery. Furthermore, resection of one layer of the muscle prevented superprojection of the underlying vessels and improved the quality of video- and photo-analysis. For a one-sided preparation, the pin length should be shortened (400-500 μm between A and B). It was found that muscle thickness can vary between rats from different strains.

Rats were weighed and subsequently anesthetized with pentobarbital. To preclude the possibility of infection, each animal received penicillin G (40.000 U i.p.). The hair at the back was removed by means of a chemical depilatory agent. An area of approximately 6 cm in

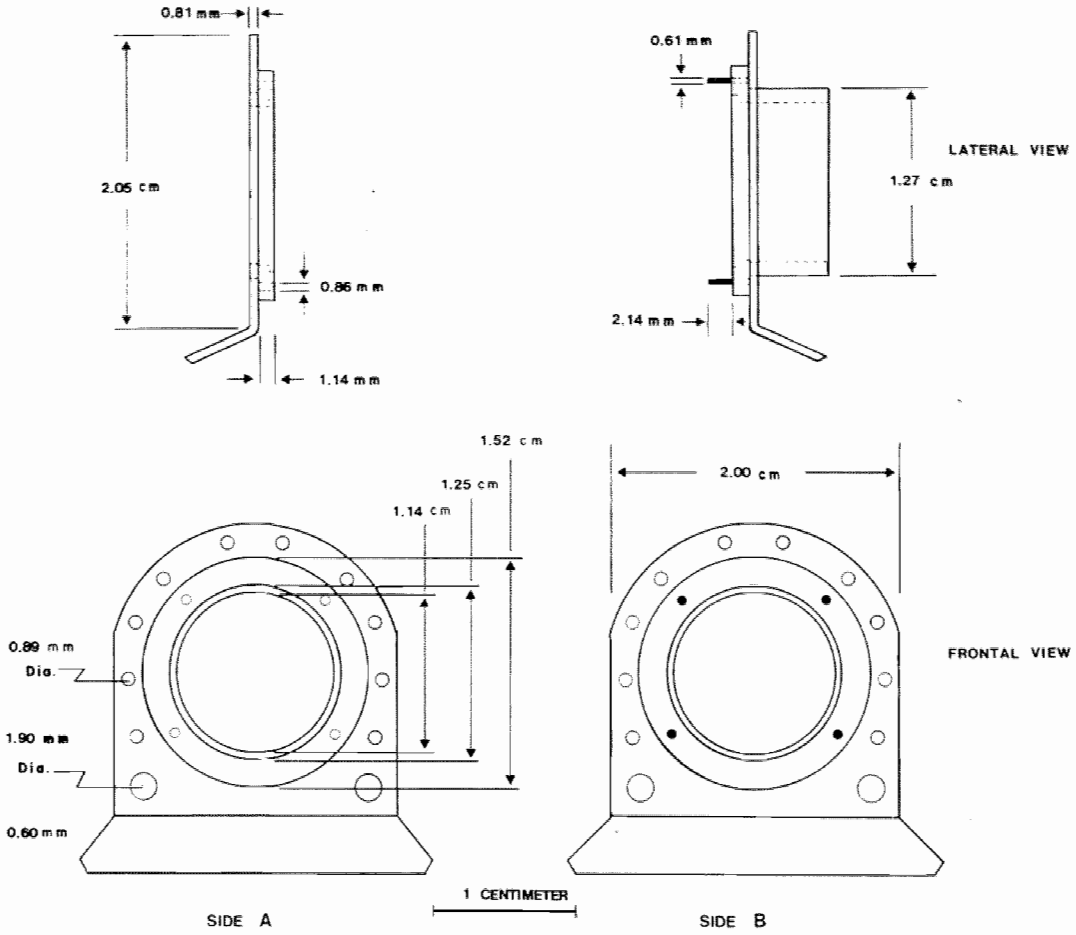

Fig. 2.1: Schematic drawing of the dorsal microcirculatory chamber according to Smith et al (1985). For a description of the chamber, see section 2.8.1

length and width beginning about 1 cm below the spine of the scapula was prepared on the dorsal side of the rat. Residues from the depilatory agent were removed. Then the area was cleansed thoroughly with an antimicrobial agent (Wiscodyne, West Chemical Products, New York, NY, USA). Surgical tools as well as the complete chamber were gas sterilized prior to surgery. The rat was placed ventral-side down on a surgery board and covered with a sterile drap to prevent contamination of the surgical field. Four 3-0 silk sutures were inserted through the skin and underlying skeletal muscle along the length of the vertebral column. The first suture was inserted approximately 1 cm from the anterior side portion of the field and the remaining 3 sutures were spaced approximately 5 mm apart. The sutures were tied to a supporting D-shaped frame. The animal was rotated on its left side and extended over the surgery board. The skin was punched and a cut was made along the edge of the outline. The skin was carefully dissected from the underlying muscle. Small bleedings were treated by applying thrombin locally (Thrombin, Thrombostat). For a one-sided preparation, a round area of the underlying muscle should be removed as well.

Then side B (right side) of the microcirculatory chamber was positioned and two marks indicating entry wounds for securing screws were made. Side B was temporarily removed to allow for cutting out the pathways of the screws and clearing of fascia and muscle by blunt dissection. Therefore side B was fitted with 1-72 Nylon screws and placed in its final position. A sterile gauze was placed over side B of the chamber. The animal was flipped over on the axis of the supporting D-frame to expose its left side. The rat was then repositioned on the board and its feet secured with tape.

Openings for the screws on the left side were cut with the scalpel blade and the screws were pushed through. The window opening on the left side was marked and cut as on the right side. The stainless steel pins of the right window were punched through the cutaneous maximus muscle with an 18 gauge needle adapter and the left window (side A) was fitted to the right window.

Air was eliminated by injecting warmed ($37^{o}C$) sterile physiological saline into the wound. Stainless steel nuts were fitted to the nylon screws and tightened to seal the chamber. The holding sutures

were removed from the D-frame and the animal was placed ventral-side down on a board to complete this part of the surgery.

Five polyfilament 4-0 sutures (Vetafil Benen, South Jackson Inc., Washington DC) were inserted around the perimeter of the chamber to anchor the skin and muscle around the window and to form a seal. A minute amount of cyano-acrylate glue was applied to each suture knot to prevent untying. A petroleum-based antibacterial ointment (Neosporin, Burroughs-Wellcome, NC, USA) was applied topically around the edges of the chamber and adjacent skin to moisturize the depilated tissue and prevent infection.

Following surgery, the animals were kept warm by placing them on a heating pad until they were fully conscious. They were housed in separate cages in a light- and temperature-controlled room (24-25^{o}C) to which they had been previously acclimatized, and maintained on regular rat chow. Between 5-7 days were required for full recovery.

The preparation was considered to be good when:

1. No major bleedings during surgery, and no microbleedings during recovery were observed;
2. No signs of inflammation such as increasing vasodilation, neovascularization or edema were found. If present, infections appeared at the top side of the preparation and could be recognized by a white-yellow discoloration; neovascularization was primarily apparent on the venous side;
3. No excessive leukocyte rolling and sticking at venular walls was present;
4. Vasomotion could be observed.

2.9 Intravital microscopy, illumination and recording systems

2.9.1 Set-up for anesthetized animals

Intravital microscopy has been developed as a technique for in-vivo studies of the microcirculation of tissues with an intact circulation and nerve supply. For the present thesis, all observations were made with a modified Leitz intravital microscope except for the experiments with conscious rats, in which a different microscope system

was employed.

The microscope and image recording equipment were mounted on two different platforms of a large aluminum frame. The entire frame rested on a platform separated from the floor for minimizing vibrations. The microscope stage was mounted on the microscope platform which allows maximum working space for the investigator. The recording system was fixed on the camera platform. The microscope was adapted to telescopic imaging (Slaaf et al, 1982). The use of a projection eye-piece (1x or 2x; p*1: focal length 250 mm; p*2: focal length 125 mm) and a transfer lens (focal length 322 mm) allows great flexibility in the vertical location of the plane of observation, while permitting rigid fixation of the recording system and transfer-lens combination. Telescopic magnification (M_{tele}) is given by the ratio of the focal length of the transfer lens (Ftl) and that of the projection eye-piece (Fpe).

$$M_{tele} = \frac{F_{tl}}{F_{pe}}$$

If transillumination was performed a long working distance condenser lens was used (L11, NA 0.60 or L20, NA 0.45). Illumination was performed according to Köhler providing highest intensity at even illumination. To minimize a possible artefactual influence of the illuminating light on the tissue under observation, the field diaphragm was set in such a way that only the tissue under observation (through monitor or eye-piece) was illuminated. Transillumination of the mesentery preparation was performed using a Tungsten lamp (60 W). Using the cremaster preparation, images of good optical quality were obtained by transillumination with a Tungsten Halogen lamp (100 W) and heat absorbing filter (Schott KG 1, Mainz, FRG) in the light path. Power was supplied by a DC-current source (Delta E30-10) for adjusting the illumination level required.

For observation of the extramural vessels of the microcirculation of the ileum, incident illumination was performed via a Leitz Ploemopak illuminator 2.1 (tube factor 1.25x) equipped with a mirror with 50% reflectance and 50% transmittance, placed at 45^{o} to the optical axis of the microscope, and a polarizer-analyzer cube (Leitz Pol-cube) for minimizing the effects of strong reflections on image

quality. The ileum was illuminated using a Xenon lamp (150 W) and a Calflex heat reflecting filter (Schott B1/K2; Mainz, FRG) in the illumination pathway. Considerable enhancement in contrast of red blood cells was achieved by placing a blue filter (Schott BG 38, Mainz, FRG) in the illumination pathway. The light level was set to the appropriate level using neutral density filters (e.g. Schott NG 5; Mainz, FRG)

Macromolecular leakage and capillary recruitment was studied using the Ploemopak 2.1 incident illuminator (1.25x tube factor) equipped with the Leitz interchangeable filter cube no I-2 (excitation filter BP 450-490; dichroic mirror RKP 510, barrier filter LP 515) in combination with a high pressure Mercury lamp (200 W) or a Xenon lamp (150 W). Both lamp houses were equipped with a Calflex heat reflecting filter.

For inspection of the preparations and mapping of the entire microvasculature, low magnification objectives (Leitz 1x, NA 0.04, Leitz 4x, NA 0.12) were employed. Individual vessels were observed using salt water immersion objectives with a high numerical aperture (SW 25, NA 0.60; SW 50, NA 1.00). In transillumination microscopy a compromise must be found between field of view and resolution. In incident fluorescence microscopy, the light gathering power, which is proportional to NA^4/M^2, becomes very important. Objectives with high NA are desirable (Slaaf et al, 1986). Because of the low light levels present in fluorescence microcopy, the regular eye-pieces (10x) were replaced by low-power eye-pieces (5x).

A rotatable mirror placed in the ray-path above the transfer lens allowed for quick shifting from one camera to another in the course of the experiment.

Images were displayed on a 12-inch video monitor (Siemens M21701) through a Grundig video camera (FA 32) with a 1-inch RCA silicon target tube (Ultricon; type 4532) and stored on a tape via video cassette recorder (Sony Betamax, type SL-C9ES). The video recorder was connected to a video timer (For-A, type VTG 33) for frame coding. For fluorescence microscopy, it was necessary to use a low-light level video camera. We used a high sensitivity silicon intensified target (SIT) camera (Bosch TYC 9A equipped with a 1-inch RCA SIT tube (type

4804). Sensitivity can be set either manually or automatically. The maximum sensitivity of this camera is about 100 times higher than that of the Ultricon camera. At highest sensitivity, the images become rather noisy which can be a serious limitation for image analysis (e.g. measurement of vessel diameter).

For photographic documentation, a motorized Nikon F2 35-mm camera was mounted in addition. The camera was loaded with a suitable Kodak Ekta-chrome slide-film.

2.9.2 Set-up for conscious animals

For studies on conscious animals, provided with a dorsal chamber (2.8.1), we used a modified Zeiss microscope (standard model 16) with the stage attached to a heavy stable plate and mounted vertically. The preparation was transilluminated using a fiber optic light guide system and a Tungsten Halogen lamp (150 W) with heat absorbing filter. For inspection and mapping of the preparation, Zeiss Plan objectives (1.25x, NA 0.04; 2.5x, NA 0.08) were employed. Individual vessels were observed with a Zeiss Plan 10x (NA 0.22) long working distance objective. The video image of the microcirculatory bed was stored on tape with a Sony Betamax recorder (SL HFR 70) for further analysis. The video recorder was also connected to a video timer for frame coding. Images were displayed on a video monitor through a 2/3 inch video camera (RCA, type TC 2500, Ca, USA). Total optical magnification in this study was 5x, 10x, and 40x (1.25x, 2.5x, and 10x objectives). For photography, a Nikon F2 35-mm camera with Kodak Ekta-chrome films were used. These resulting slides served as documentation.

2.10 Microvascular measurements

The tools for quantifying microvascular alterations have been refined in the last decade. With electronic methods, diameter and velocity measurements can be obtained from the microscope images or from the recorded video images in case of off-line analysis.

2.10.1 Diameter measurements

Diameter measurements were performed off-line with an image-shearing device which was built in our own workshop (Intaglietta and Tompkins, 1973). On the monitor, the video image could be rotated electronically to position the vessel vertically. The image of the blood vessel was then sheared along a line perpendicular to the axis of the vessel. If properly calibrated, the spatial translation required to align the opposite edges of the inner vessel wall is a direct measure of the diameter of the blood vessel. The mean vascular diameter was defined as the linear average of this distance. The displacement represents the inner vessel diameter. By aligning vessel walls, rather than measuring the diameter at one site, the accuracy of the measurement is in principle better than the optical resolution of the microscope.

Several factors may limit the accuracy and precision of the diameter measurements. One problem is to accurately localize the lumenal side of the vessel wall. A thin layer of reduced light intensity, due to a locally reduced number of red blood cells exists near the vessel wall. This layer can easily be mistaken for the intima of the vessel wall.

A second complication is related to the shape of the vessel itself. Arterioles can be contorted under varying conditions of vascular tone (Proctor et al, 1984) with invaginations of the vessel wall into the lumen and therefore do not form a perfect cylinder. To assess the vascular diameter, focussing of the microscope was done by bringing into focus the widest possible image of the vessel.

Dynamic diameter changes as occur in arterioles exhibiting vasomotion were always analyzed with a shearing monitor and its output was recorded on a physiological recorder. In some experiments where vasomotion was absent, a vernier calliper was used as a reliable alternative to measure the static vessel diameter.

2.10.2 Measurement of red blood cell velocity

At the present time, no methods are available to directly measure volume flow in microvessels. Most electronic methods currently in use rely on the assessment of the velocity of passing red blood

cells. Red blood cells are by far the most numerous cells in the blood and are an good optical marker of flow. It is beyond the scope of this thesis to discuss the different existing methods to measure red blood cell velocity in microvessels (e.g. the dual-slit method, the spatial filtering (BDO) method, or laser Doppler velocimetry). The reader is referred to recent surveys (Slaaf et al, 1984, 1986).

For the present thesis, velocity measurements were performed with the dual-slit method and a velocity tracking correlator (both from Instrumentation for Physiology and Medicin Inc (IPM), San Diego, CA, USA).

The circulating RBCs have a certain distribution over the cross-sectional area of the microvessel and travel at different velocities. Applying regular microscopic techniques, the image constitutes a place- and time-dependent light intensity signal. Sensors placed on the intermediate image, collect time-dependent signals from these spots. By processing these signals in an appropriate way a measure of RBC velocity is obtained. The passing RBCs produce characteristic peaks in the upstream and downstream signals of the photodiodes. These signals are cross-correlated in order to determine their average delay. This delay (Δt) is inversely proportional to the RBC velocity. The output of the velocity tracking correlator, however, is directly proportional to $1/\Delta t$. Thus, inversion is avoided. After appropriate calibration of the spacing of the sensors, the velocity tracking correlator directly provides velocity. Changes in velocity should be tracked by the correlator. Within a certain range of rate of changes in velocity, velocities are automatically tracked. Velocity is continuously recorded on a Schwarzer strip chart recorder. With the dual-slit method, dynamic velocity changes can be tracked over a range of a factor of 10 between velocities of 0.05-50 mm/s.

The two fibers in the photodiode sensor head have an actual center to center spacing of about 0.95 mm and are mounted in front of the sensitive surface area of the video camera and are seen as one composite "black needle" on the video monitor. This allows for positioning of the sensors above the image of the vessel close to its centerline. This spacing should be compared to the magnified microscope image, so the spacing $d=0.50/M$ µm where M is the total optical

magnification. Optimal spacing is between 5-20 μm, resulting in a required optical magnification between 50 and 200 times.

The dual-slit method relies on changes in optical density. It is essential that changes in illumination power are minimized. To this end, a Tungsten lamp was connected to a stabilized DC source. The Tungsten lamp provides appropriate contrast beween red blood cells and surrounding plasma and thus a sufficient signal-to-noise ratio. Especially in larger vessels, contrast enhancement by using blue filtering has to be avoided since it results in very weak correllograms. Since the photo-optic pick-up unit is positioned a few centimers in front of the sensitive surface camera signal-to-noise ratios, and thus velocity measurements, could be improved by slight defocussing. However, a draw- back of this is that the simultaneous measurement of velocity and vascular diameter is not feasible anymore.

The relationship between velocity, as assessed by the dual-slit method with the sensor positioned in the centerline of the vessel, and volume flow assessed by Baker and Wayland (1974) was calculated to be 1.6. This factor however may be affected by changes in vascular tone, the size of a given blood vessel or the local hematocrit (Pittman and Ellsworth, 1986). In addition, the flow profile in vessels with laminar flow is not parabolic but blunted and varies with the vessel diameter (Tangelder et al, 1986). Moreover, the centerline velocity depends on the width of the sensor size relative to the vessel diameter (Slaaf et al, 1986). Calculated volume blood flow based on diameter and red blood cell center-line velocity (V_{rbc}) measurements using the dual-slit cross-correlation method can only yield estimates and therefore in the present studies only V_{rbc} was used.

2.11 Statistical analysis

All data are presented as means ± SEM, unless indicated otherwise. In chapter 3, group comparison between hypertensive and normotensive animals were made with the Mann-Whitney U-test (Johnson, 1984) using the minitab program (Ryan et al, 1985) and a VAX computer. Base-line values for MAP and HR in 3 groups in chapter 6 were compared

with a one-way analysis of variance and a modified t-test (Wallenstein et al, 1980). Dose-response curves were compared with one-way analysis of variance as described by Zerbe (1979) using a VAX computer or ANOVA with repeated measures. Significance was determined at the 95% confidence level.

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3. REGULATION OF PERFUSION OF SKELETAL MUSCLE DURING THE EARLY PHASE OF SPONTANEOUS HYPERTENSION IN RATS: A FUNCTIONAL MORPHOMETRIC STUDY

3.1 Introduction

Spontaneous hypertension in the rat is characterized by an increased vascular resistance of virtually every organ system (Nishiyama, 1976; Zweifach, 1983; Evenwel et al, 1983; Bohlen, 1986). The increased vascular resistance in spontaneous hypertension is caused by alterations at the arteriolar level as has been shown for various tissues (see chapter 1).

Maturation is associated with growth and development of the microvascular bed. The volume of tissues increases as well, but to a greater extent than microvascular growth. As a consequence, vascular densities will diminish as a function of age (Sarelius et al, 1981). An age-related hampered vascular outgrowth , i.e. rarefaction, may account for an increased resistance and blood pressure in SHR (Bohlen, 1986). Furthermore, during the developmental phase of spontaneous hypertension vessel lumen size may decrease. Until now evidence for a reduced vessel lumen has been obtained in in-vitro studies or in isolated perfused organs but not in direct intravital microscopic studies.

In both WKY and SHR peripheral resistance increases with age. At the age of 5-6 weeks there are no statistically significant differences between the peripheral resistance in WKY and SHR (Smith and Hutchins, 1979; Evenwel et al, 1983) but cardiac output is unevenly distributed in SHR. The skin and skeletal muscle vascular beds receive an enhanced flow. At the same age, in SHR peripheral resistance and blood pressure start to rise progressively and stabilize at 7-9 weeks (Evenwel et al, 1983). In addition, the peripheral resistance following complete vasodilation is also increased at 9 weeks (Prewitt et al, 1982). The distribution of cardiac output is normalized at this age.

Few studies attempted to investigate the distribution and regulation of blood flow in the early phase of hypertension. Since striated muscle is responsible for the (ultimate) bulk of the increase in peri-

pheral resistance the present study is focussed on local flow regulatory mechanisms in striated muscle in the resting state and following vasodilation.

Vessel diameters were measured in the control state and following maximal vasodilation, as achieved by adenosine, a potent endogeneous vasodilator (Proctor, 1985). From these data, vessel lumen size and micro-vascular tone at several arteriolar levels were assessed. Micro-vascular tone was expressed in the form of a non-dimensional tone as the difference between steady-state and dilated diameter (following adenosine treatment) divided by the dilated diameter (Schmid-Schönbein et al, 1987).

Under resting conditions, differences with regard to vascular densities need not to be manifest because only a fraction of functionally available vessels are perfused. In order to assess the flow carrying capacity, arteriolar densities at several levels within the microvascular bed were determined in the resting state and following vasodilation where all functionally available vessels are perfused. Furthermore, the density of plasma perfused capillaries was established by means of fluorescence microscopy.

The study was performed on the cremaster muscle of 5-6 weeks old hypertensive rats and age-matched normotensive control rats. This age was chosen because spontaneous hypertension is developing rapidly.

3.2 Experimental protocol

3.2.1 Animals

Young SHR of 5-6 weeks and age-matched WKY rats were anesthetized with alpha-chloralose urethane (see section 2.1) and the cremaster muscle prepared for intravital microscopic observations as described in detail in sections 2.7.3 and 2.9.1. Central hemodynamic parameters, mean arterial pressure (MAP) and heart rate (HR), were measured via the carotid artery (section 2.3). The jugular vein was used for systemic injections of FITC-BSA (section 2.3). For a general lay-out of the experimental set-up see fig. 3.1.

Following equilibration, vessels were classified based on their

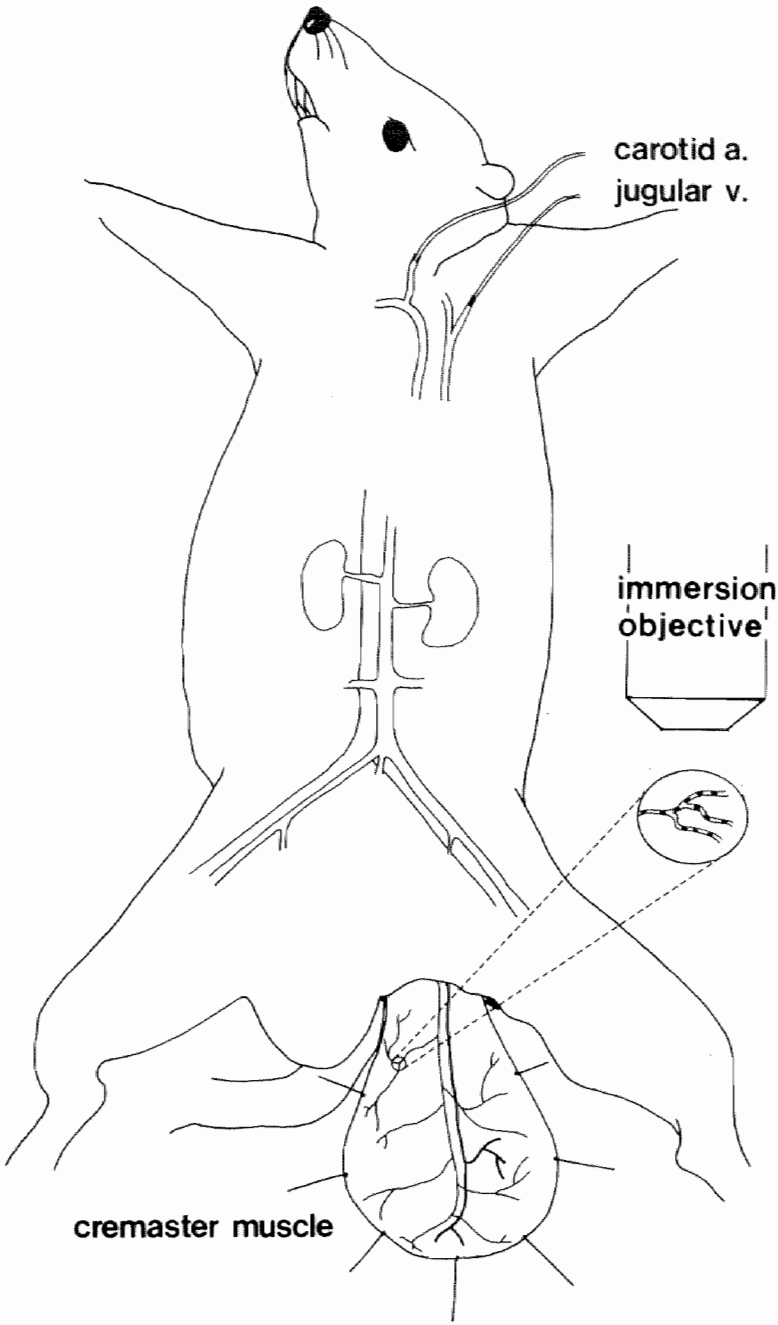

Fig. 3.1: General lay-out of the experimental set-up for the cremaster muscle.

functional branching order.

3.2.2 Microvascular terminology

Microvascular topology should classify the vessels according to their function in distributing the flow over the organ. At rest, a certain distribution of flow occurs. When vasodilated a different partition of flow can be observed. Topology should be such that a separation in different classes allows for maximal recognition of the different functions.

Terminology in this study was based on the identification of different vessel types by their location in the network relative to their branching order and function. This scheme was first applied to the cremaster muscle by Hutchins and Darnell (1974). A representative version of a microcirculatory network with well defined characteristics is shown in fig. 3.2.

In the cremaster muscle in the middle of the preparation there is only one straight running perfusing arteriole which was defined as first order arteriole (A1). All side-branches of the single A1 vessel were defined as second order vessels (A2). Along the second order vessels, Y-shaped branchings with equal diameters or head-on interconnections with similar large arterioles were all classified as being A2. Lateral off-shoots of second order vessels with smaller diameters were classified as third order vessels (A3). All side-branches of A3 vessels with distinctly smaller diameters were classified as fourth order vessels (A4). Since fourth order arterioles were precapillary arterioles, no further downstream classification scheme was applied.

3.2.3 Arteriolar diameters and recruitment

The entire muscle was documented using a Leitz 1x objective in combination with its Leitz condenser lens to study the gross angioarchitecture of the muscle in particular the A1 and A2 vessels.

The experimental protocol was divided into 3 subgroups: (1) The entire A1 vessel and all its side-branches (A2) close to their branching point were observed via a Leitz 4x objective and overlapping recordings were made for off-line measurements of vessel diameter and segment length between two side-branches; (2) In the anteromedial part

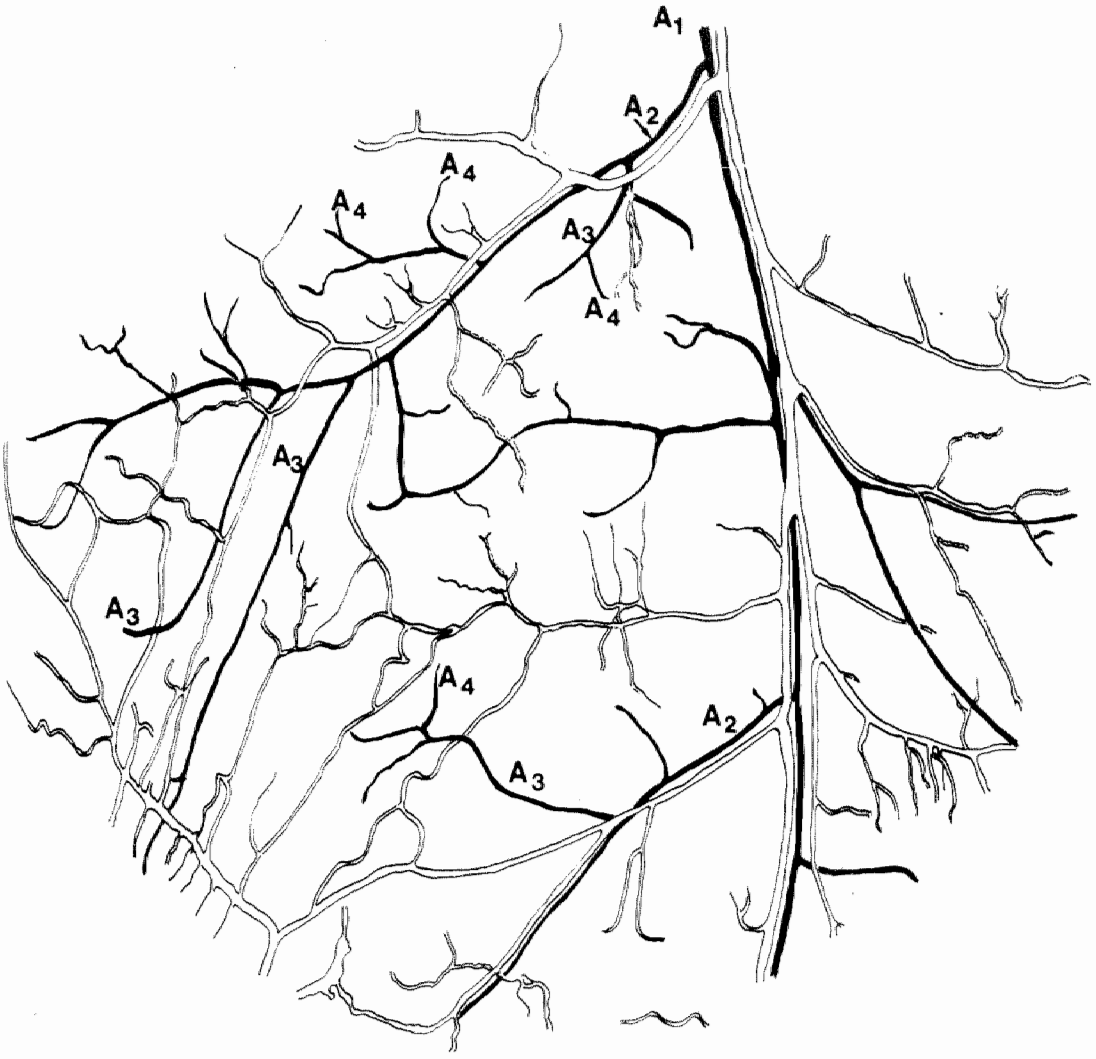

Fig. 3.2: Microvasculature of the cremaster muscle. The alphanumerical vessel classification refers to first (A_1), second (A_2), third (A_3) and fourth order (A_4) arterioles.

of the muscle with high transparency (see section 2.7.3), one A2 vessel was selected. Image recordings were made with a Leitz 4x objective. A hand-drawn map was made for quick orientation during the course of the experiment. All perfused side-branches were documented with a salt-water immersion objective (SW 25); (3) One arteriole among the A3 vessels which branch off from the A2 vessel was selected and meticulously studied for the occurrence of side-branches (A4) as evidenced by flowing RBCs. Selection of the A3 vessel was solely based on clear distinction of the margins of the vessel wall over a distance of at least 1500 µm. Many arterioles close to the surface of the muscle allow for proper diameter analysis and detection of all their side-branches (A4). Selection of an A3 close to the edge of the preparation was avoided because (total) microvascular blood flow tends to be higher in traumatized areas altered by dissection (Lindbom et al, 1982; Proctor and Busija, 1985).

After completion of this part of the protocol, adenosine was added in the superfusate to achieve a final concentration of 10^{-3} M to cause maximal vasodilation. The vasodilation was considered to be maximal since in pilot-experiments no additional increase in diameter was observed following addition of 0.2 mg/ml sodium nitroprusside to the adenosine superfusate. The entire protocol (1-3) was repeated 15 min following superfusion with adenosine. In a small number of experiments recruited A3 vessels in WKY and SHR were documented for analysis and number of side-branches and segment lengths.

During the recordings one investigator viewed directly through the microscope eye-pieces and counted the number of vessels. The second investigator took care of proper video-documentation and of focussing at several tissue depths for optimal images on the monitor. Diameter analysis (section 2.10.1) was performed off-line. The dilating capacity of arterioles was assessed according to Schmid-Schönbein et al (1987).

Vessel segment lengths beween side-branches and total vessel length measurements were made off-line from overlapping video-images. Total vessel length was always determined in the vasodilated state. Length of first order arterioles was determined by measuring the length between the site of the first off-spring until its last side-

branch. The last segment was determined by its last side-branch which could be detected during vasodilation and contained flowing red blood cells. Length measurements were performed with a calibrated vernier calliper. The number of side-branches of each vessel category was normalized by calculating the mean interdistance. The number of side-branches per unit of vessel length was converted into mean distance, i.e. interdistance.

3.2.4 Involvement of arteriolar arcades

In addition in 5 experiments, all A3 vessels fed by one A2-vessel were examined over their full length to determine whether they were part of an arcade, that is whether they formed at their end a second connection with the A2, during both control and vasodilation. This was also carefully examined for recruited arterioles which were directly fed by the A1 vessel.

3.2.5 Capillary diameter and recruitment

During the third part of the protocol (section 3.2.1), in the downstream area of A4 vessels capillaries in 6 WKY and 6 SHR were selected within a shallow depth of the tissue. Capillaries were observed and documented using an SW50 objective in bright-field microscopy. Recordings for diameter analysis were made in the control state and following adenosine. Diameters were measured with a vernier calliper.

Capillary recruitment was studied using fluorescence microscopy (section 2.9). Capillaries were visualized after a slow intravenous injection of FITC-BSA Sigma (200 mg/kg). Along an A2 vessel, 10 areas were selected where capillary flow was supplied by the A2. Video-recordings were made for off-line analysis of the proper capillary bed and care was taken to select an area where no larger vessels could interfere with obscured an accurate measurement of capillary lengths. For each recording site, the XY-coordinates of the microscope stage were determined with a digital microposition sensor and documented (Heidenhain, GmbH, Traunheut, W-Germany). For accurate reference of the recording site, a second video casette recorder (VCR) was used to record the site of measurement and to search for the original area of

measurement. When this part of the experiment was completed, the preparation was treated with vasodilators and the protocol was repeated.

In pilot-experiments it was found that topically applied adenosine induced plasma leakage, as evidenced by the extravascular appearance of FITC-BSA, and consequently images were hardly suitable for analysis. Leakage of FITC, however, could effectively be reduced by a 10-15 min pretreatment with a beta-2 agonist terbutaline (Bricanyl^{R}, Astra Chemicals Benelux, Rijswijk, The Netherlands; 0.5 mg/ml). Following pretreatment with terbutaline (final concentration in superfusate 250 µg/l), the experiment was continued by superfusion with adenosine (10^{-3} M) and terbutaline. Both agents were dissolved in the Tyrode's solution. Neither treatment or combination of terbutaline with adenosine produced any measurable change in arterial pressure.

All video-recordings of capillaries were made during a 1-2 min period. By slowly and carefully focussing at different tissue depths, all capillaries were included.

At the end of the experiment in 6 WKY and 6 SHR, tissue thickness of the anteromedial part of the muscle was measured by focussing on the upper surface of the muscle and then on the lower side using the fine adjustment micrometer scale, always using a salt-water immersion objective (SW25). By slowly replaying and stopping the video-tape capillaries of all tissue depths were analyzed. Total capillary length of each area (200 x 250 µm) was determined twice with a calibrated curvimeter and the mean value of each area was used for further data analysis. Of each animal, the mean value of 10 areas was determined.

Capillary density was expressed as the total capillary length (units) of surface area and per volume ratio.

3.2.6 Statistical analysis

All data are presented as means $\pm$ SEM. N = number of animals. To test for differences in blood pressure, heart rate, age and body weight between the two groups, the Student's t-test was used. For comparison of vessel diameter and density between the hypertensive and normotensive groups, the non-parametric Mann-Whitney U-test was used. Values of p less than 0.05 were regarded as significant.

3.3 Results

3.3.1 General findings

Experiments were successfully performed on 14 WKY and 14 SHR. The MAP, HR, age and body weight values of WKY's and SHR's are listed in table 3.1. MAP in the hypertensive animals was only modestly increased (on the average by 14 mm Hg) as compared to the normotensive control rats. Differences between these groups were statistically significant ($p<0.05$). The hypertensive rats had a higher heart rate ($p<0.01$). All rats used in this study were 5-6 weeks old and had body weights of approximately 105 grams. No statistically significant differences between the groups of hypertensive and normotensive rats were found, as far as the values of the two latter parameters are concerned.

Table 3.1: Group comparison between WKY and SHR.

	WKY	n	SHR	n	
MAP (mm Hg)	89 ±3.0	14	103 ±3.9	14	$p<0.01$
HR (bpm)	343 ±12.0	14	380 ±16.0	14	$p<0.05$
age (days)	38.1±1.1	14	36.1±0.9	14	n.s.
body weight (grams)	106 ±3.4	14	105 ±5.9	14	n.s.

3.3.2 Microvascular geometry

Arteriolar branching patterns were consistent. In the resting situation, each preparation was perfused by only one major arteriole (A1). The A1 vessel branches usually into 6-10 straight running A2 vessels, which distribute blood over the muscle and are coiled in the distal part of the preparation. The vascular branching pattern of the A1 vessel can be compared with an asymmetrically bifurcating tree.

Five to 8 A3 vessels branch off perpendicularly from the A2 arterioles and supply a restricted area with blood. Three to 6 A4

Table 3.2: Arteriolar diameters in µm.

	Control			Vasodilation			Tone (%)		
	WKY		SHR	WKY		SHR	WKY		SHR
A1	71.6±2.4	n.s.	75.8±3.2	82.8±2.8	n.s.	85.0±3.1	13.5±3.4	n.s.	10.8±3.0
A2	48.9±1.1	n.s.	48.7±1.1	56.9±1.2	n.s.	56.9±1.1	14.1±2.5	n.s.	14.4±1.6
A3 (perfused at rest)	18.5±0.9	$p<0.05$	21.4±0.9	25.4±1.0	$p<0.05$	28.9±1.0	27.2±2.0	n.s.	26.0±1.3
A3 recruited (A2)	-		-	19.4±2.6	n.s.	18.1±0.9	-		-
A3 recruited (A1)	-		-	28.3±1.1	n.s.	28.1±1.7	-		-
A4 (perfused at rest)	9.8±9.3	n.s.	10.0±0.4	15.8±0.4	n.s.	16.2±0.5	38.0±1.2	n.s.	38.3±1.5
A4 recruited	-		-	13.6±0.4	n.s.	12.8±0.7	-		-

n.s.: not significant
Vascular tone expressed as percentage and computed according to Schmid-Schönbein et al (1987)

arterioles branch off perpendicularly or obliquely from A3 vessels. Finally these A4 vessels give off the capillaries which form a capillary network. Within a layer, capillaries can be nicely aligned or form a dense meshwork. Sometimes several layers can be distinguished.

3.3.3 Arteriolar diameters

In table 3.2 the diameters in the control state and following dilation of arterioles which were perfused in the control state are shown. A statistically significant difference was only found for third order vessels, the diameter of which was even slightly larger in SHRs. Topical administration of adenosine caused all vessels in both groups to increase in diameter. Again except for the A3 arterioles no significant differences were observed between the diameter in the SHR and the WKY group. Vasodilation was relatively stronger in smaller arterioles.

The dilating capacity increased toward the capillary bed. Third and fourth order vessels exhibited the highest increase in diameter.

At several levels within the microvascular bed recruitment of arterioles was observed in both WKYs and SHRs. Recruited A3 vessels had smaller diameters than comparable arterioles already perfused in the control state ($p<0.01$) (see table 3.2). This was also found for the recruited A4 vessels ($p<0.01$).

Following vasodilation the number of arterioles perfused by the first order arteriole increased as well. Based on their maximum diameter these vessels were classified as A3 vessels because no differences were noticed with "true" A3 vessels (table 3.2).

3.3.4 Arteriolar recruitment

Each preparation was fed by one A1 vessel (table 3.3) both in the resting situation and following vasodilation. The number of perfused A1 and A2 vessels did not differ between WKY and SHR (table 3.3). As a consequence the interdistance of A2 vessels between SHR and WKY did not differ statistically in the resting state and following vasodilation. In the resting situation, the number of A3 vessels per A2 was smaller in SHR as compared to WKY. Consequently, the mean interdistance in SHR was longer ($p<0.05$) (table 3.3).

Table 3.3: Number of arterioles and interdistance as its normalized length for each type of parent vessel.
n.s. = not significant
n = number of animals

	WKY	n	SHR	n	
Number of A1 vessels					
Control	1.0 ±0	14	1.0 ±0	14	
Vasodilated	1.0 ±0	14	1.0 ±0	14	
Increase in perfused A1 vessels	0	14	0	14	
Number of A2 vessels					
Control	7.5 ±0.7	12	8.8 ±0.5	14	n.s.
Vasodilated	7.5 ±0.7	12	8.8 ±0.5	14	n.s.
Increase in perfused A2 vessels	0	12	0	14	
Interdistance (µm)					
Control	2064±119	12	1885±142	14	n.s.
Vasodilated	2064±119	12	1885±142	14	n.s.
Change in inter-distance	0	12	0	12	n.s.
Number of A3 vessels					
Control	7.9 ±1.1	12	5.2 ±0.5	14	p<0.05
Vasodilated	13.0±1.4	12	6.6 ±0.5	14	p<0.01
Increase in perfused A3 vessels	5.1 ±0.9	12	1.4 ±0.3	14	p<0.05
Interdistance (µm)					
Control	1932±340	12	2800±268	14	p<0.05
Vasodilated	1135±222	12	2168±186	14	p<0.01
Change in inter-distance	797±177	12	632±175	14	p<0.05
Number of A4 vessels					
Control	5.9 ±0.9	11	3.3 ±0.5	10	p<0.05
Vasodilated	9.1 ±0.8	11	4.4 ±0.5	10	p<0.01
Increase in perfused A4 vessels	3.2 ±0.1	11	1.1 ±0.2	10	p<0.05
Interdistance (µm)					
Control	488 ±72	11	880 ±145	10	p<0.05
Vasodilated	304 ±44	11	616 ±100	10	p<0.05
Change in inter-distance	184±39	11	264±73	10	p<0.05

Following vasodilation the number of A3 vessels perfused increased. This was seen in both SHR and WKY. The magnitude of the increase in the number of A3 perfused was less in the SHR ($p<0.05$). The discrepancies between WKY and SHR in interdistance became more pronounced (table 3.3). Of the functionally available A3 vessels approximately 39% was not perfused in WKY and only 26% in SHR.

The same pattern was found for the A4 vessels. In the control situation, the number of A4 vessel was less and the mean interdistance was longer in SHR than in WKY (table 3.3). In the vasodilated state, a smaller number of A4 vessels was recruited in SHR as compared to WKY. Of the functionally availabe vessels at rest 35% of the A4 vessels was not perfused in the WKY and only 25% in the SHR.

The recruited arterioles (A3) with A2 as parent vessel were further examined. The interdistance of off-shoots of A2 vessels was: WKY: 299±39 μm (n=5) and SHR: 533±37 μm (n=12), respectively. Their microvascular characteristics (interdistance) did not differ from the control group and can thus be regarded as identical.

The absolute number of recruited "A3 vessels" fed by an A1 vessel was less in SHR as compared to WKY, but did not reach the level of significance (WKY: 2.2±0.2, n=26; SHR: 1.5±0.5, n=19). The interdistances of these vessels in the SHR were comparable with their corresponding control group (A3 vessels perfused at rest).

3.3.5 Arteriolar arcades

The number of third-order arterioles interconnected to form an arcading system was small. In both SHR and WKY only one A3 vessel appeared to be part of an arcade in the control situation (number of A3 analyzed: 20 and 25). In the SHR group it was noted only once that a recruited arteriole opened up to an arcading network. Arteriolar arcades were never observed at the level of fourth order arterioles.

3.3.6 Capillary diameters and recruitment

In the control situation the capillary diameters in WKY and SHR did not reveal any statistically significant differences (table 3.4). Adenosine did not increase diameters in both strains.

Tabel 3.4: Capillary diameter (μm), capillary length (mm/mm^2) and cremaster muscle thickness (μm) in 6 WKY and 6 SHR in the resting situation and following topical treatment with adenosine (final concentration: 10^{-3} M).

	WKY		SHR
Capillary diameter (μm) (control situation)	3.9±0.4	n.s.	4.0±0.5
Capillary diameter (μm) (vasodilation)	4.0±0.4	n.s.	4.0±0.4
Capillary length (mm/mm^2) (control situation)	44.9±1.7	p<0.01	33.1±1.2
Capillary length (mm/mm^2) (vasodilation)	45.8±1.7	p<0.01	34.6±1.4
Muscle thickness (μm)	259.3±5.7	n.s.	251.7±11.2

In table 3.4 the capillary density (expressed as mm capillary length/ mm^2 tissue surface area) in WKY and SHR are presented. Muscle thickness of WKY and SHR did not differ statistically (WKY: 259.3 ± 5.7 μm vs. SHR: 251.7 ± 11.2 μm). In the control state in SHR the capillary density was approximately 26% less than in WKY. Since capillary density did not change in the vasodilated state, no recruitment could be determined.

3.4. Discussion

3.4.1 General

This study was designed to quantitate functional and structural differences in vasculature between WKY and SHR rats in the early phase of spontaneous hypertension by means of functional morphometry. In the control situation small but statistically significant differences between WKY and SHR were observed. In the control situation the SHR have a lower flow carrying capacity and exchange surface area. In the vasodilated state equivalent with an increased demand of flow, SHR

have less capacity to dilate and to recruit. Only at the capillary level, no changes were observed.

3.4.2 Microvascular terminology

The vessel classification used in this study reflects the functional determinants of the cremaster muscle. First and second order arterioles (A1 and A2) have a transport function and have a relatively low dilating capacity with large diameters. Third and fourth order arterioles (A3 and A4) have a regulatory function with a high dilating capacity and small diameters. In a number of A3 and A4 vessels a complete temporal closure of the vessel lumen occurs. These vessels can be recruited.

The functional significance of arteriolar arcades in regulation of flow seems to be limited because they are rarely observed for third order arterioles and are even absent for fourth order arterioles.

3.4.3 Arteriolar diameter

In the present study during the control state and following vasodilation, diameters of arterioles of 5-6 week old SHR are not significantly different from those in WKY (see table 3.2). The data presented by Bohlen et al (1977), Bohlen and Lobach (1978), Dusseau and Hutchins (1979), and Chen et al (1981) agree with our findings. Only a single report showed a higher vascular resistance corresponding with an increased dilating capacity (Roy and Mayrovitz, 1982), while Hutchins and Darnell (1974) reported even an increased arteriolar diameter in young SHR. Our findings may be extrapolated to skeletal muscle preparations like the rat gracilis (Prewitt et al, 1982) and the spinotrapezius muscle (Zweifach et al, 1981) revealing no evidence for an increased vasodilating capacity at an early age.

Comparable vessel types of the age-matched rats in this study did not reveal changes in maximal diameter in the vasodilated state except for the A3 vessels, showing larger diameters. This is at variance with the hypothesis proposed by Folkow (1983) suggesting that spontaneous hypertension is characterized by an increased vessel wall and hypertrophy reduces the vessel lumen size following maximal vasodilation. Our evidence is, however, indirect because we did not meas-

ure wall thickness.

3.4.4 Arteriolar recruitment

A low number of arterioles can have either an anatomical or functional cause (Zweifach, 1983). Functional rarefaction is characterized by an increased number of arterioles which were temporarily non-perfused, and has been demonstrated for the cremaster (Chen et al, 1981) and gracilis muscle (Prewitt et al, 1984). Structural rarefaction is characterized by a diminished number of arterioles functionally available following maximal vasodilation.

Most studies focussing on functional and structural alterations of the microvasculature of the cremaster muscle used stereological techniques by counting the number of intersections (Chen et al, 1981) or analyzed microvascular networks from photomontages by measuring the length of each individual vessel per unit surface area (Hutchins and Darnell, 1974; Engelson et al, 1986).

We have chosen for functional morphometry by measuring the distances between side-branches of the same vessel order level along the representative parts of the arteriolar tree and expressing the distances per unit vessel length. Our method is preferable because it allows for discrimination between the number and the length of parallel conductance vessels because the branching pattern is known. Although in the cremaster muscle arteriolar arcades do not play a significant role in regulation of tissue perfusion these can in principle be distinguished as well.

From the absence of recruitment of A1 and A2 vessels together with their low dilating capacity, it can be concluded that A1 and A2 vessels have a transport function. The lack of difference between SHR and WKY indicates a normal transport function in SHR.

The flow carrying capacity in the terminal portion of the vascular bed is lower in SHR as compared to WKY. The arteriolar reserve determined when all arterioles were functionally available appeared to be lowered in SHR. The regulation of perfusion is primarily restricted to the terminal part of the circulation, since arteriolar recruitment was only observed for A3 and A4 vessels.

The low number of A3 and A4 vessels in the SHR as compared to

WKY may account for an additional increase in resistance and/or rise of arteriolar blood pressure.

Our study agrees with a study published by Hutchins et al (1982) showing decreased arteriolar densities in the cremaster muscle of young rats. This is not unique for the cremaster muscle and has been reported for the gracilis muscle (Prewitt, 1982; Henrich and Hertel, 1982). Our study is partly at variance with studies of Chen et al (1981) which demonstrated besides an anatomical also a functional rarefaction at the same age suggesting an increased dilating capacity.

3.4.5 Capillary diameter and density

The presented results are in agreement with previous studies performed on skeletal muscle preparations (Chen et al, 1981; Prewitt et al, 1982) showing no difference between WKY and SHR in capillary diameter.

In the control situation in SHR, the capillary exchange surface area was clearly diminished and capillary rarefaction was present. No capillary recruitment occurred. This means that all capillaries functionally available are perfused in the control state of the tissue and that arterioles do not temporarily shut off groups of capillaries. Capillaries apparently form one dense network with dense mutual interconnections. The capillary network has a multiple input system. Hence, no increased number of perfused capillaries was observed.

Although others (Chen et al, 1981) have shown that capillary recruitment does occur at this age in SHR, the differences may be easily attributed to methodological differences. In the present study, capillaries were visualized by means of fluorescence microscopy and a plasma marker and not by the presence of flowing RBCs as observed in bright-field microscopy. Furthermore, microvascular observations are in most cases restricted to a fairly shallow depth of tissue and capillaries situated in deeper layers of the tissue may be overlooked when using brigh-field microscopy.

Our results are in accordance with observations in the cremaster muscle of young 5-week old hamsters where maximal vasodilatation failed to increase capillary density (Sarelius et al, 1981).

Capillary rarefaction was also observed in the gracilis muscle

(Prewitt et al, 1984), but contrary to the findings in our study, no capillary rarefaction could be determined in the spinotrapezius muscle (Gray, 1982) in both young and mature SHR as compared to WKY. Whether this is a pecularity of the spinotrapezius muscle has to be assessed.

In conclusion, in the young hypertensive rat with a rapidly increasing blood pressure both the number of perfused arterioles at rest as well as the functional arteriolar reserve was diminished. This was associated with an equally affected decreased capillary density. No evidence was obtained for an increased dilating capacity or smaller diameters in the passive state in spontaneous hypertension. The primary defect underlying the increased resistance and thus the progressive rise in blood pressure is a structural alteration of the vascular architecture probably due to a diminished vascular growth.

3.5 References

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4. CUTANEOUS MUSCLE MICROCIRCULATION IN ADULT CONSCIOUS NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

4.1 Introduction

Microvascular alterations of resistance vessels of nearly every organ system and in particular skeletal muscle may contribute to a considerable degree to the increased peripheral resistance in spontaneous hypertension (Bohlen, 1986). The information concerning the changes of the microvasculature of SHR as compared to WKY are mainly obtained by intravital microscopic observations of acute preparations requiring surgical manipulations with general anesthetics (Hutchins and Darnell, 1975; Henrich et al, 1978; Chen et al, 1981; Prewitt et al, 1982; Bohlen, 1983). These interventions may disturb normal physiology and vascular smooth muscle reactivity.

A depressent effect of general anesthetics on vascular smooth muscle tone has been reported for both macro- and microcirculation (Smith and Hutchins, 1980; De Lano and Zweifach, 1981; Faber et al, 1982; Colantuoni et al, 1984). Furthermore, in several microvascular studies using skeletal muscle preparations, different anesthetics or combinations of anesthetics are used complicating comparison of experimental data.

Microvascular data obtained from muscle preparations in conscious SHR and WKY are scarce. For the present study, we used the dorsal microcirculatory chamber as a technique for microscopic observations of the cutaneous maximus muscle in conscious rats. Anatomical analysis of this muscle revealed predominantly fast-twitched red fibers (Type IIA) and therefore this muscle can be regarded as striated muscle (Smith et al, 1985). Vascular resistance in skeletal muscle is increased during the established phase of hypertension and is responsible for the bulk of the increased peripheral resistance (Evenwel et al, 1983; Iriuchijima, 1983; Mueller, 1983). In chapter 3, it was shown that an enhanced arteriolar dilating capacity is not a primary step in the development of spontaneous hypertension. There is growing evidence that during the maintenance phase of spontaneous hypertension (larger) arterioles and/or arteries do exhibit smaller

diameters (Bohlen, 1986; Schmid-Schönbein et al, 1987; Miller et al, 1987) and contribute to vascular resistance. Microvascular observations of various skeletal muscles of anesthetized rats did not consistently demonstrate a reduced lumen of arteriolar resistance vessels in spontaneous hypertension.

Therefore, in this study, comparable types of vessels in adult conscious WKY and SHR were analyzed. In addition, we looked for the presence of vasomotion, a phenomenon which can be regarded as normal in the intact tissue (Funk et al, 1983; Colantuoni et al, 1984; Mulvany, 1983) and may play a role in the regulation of tissue perfusion (Tangelder et al, 1984) and in determining vascular resistance (Funk et al, 1983). A decreased amplitude of vasomotion and an increased calculated vascular resistance of arterioles were demonstrated for the spinotrapezius muscle in SHR (Borders and Zweifach, 1979; Borders, 1980).

4.2 Experimental protocol

Rats (SHR and WKY) were provided with the dorsal microcirculatory chamber as described in detail in section 2.8.1. Approximately one week was allowed for full recovery. At the experimental day, rats (age 10-12 weeks) were placed in the rodent restrainer for in-vivo microscopy. Details about the microscope, which was rotated 90^{o}, and recording system can be found in section 2.9.1. Rats were loosely restrained. The restrainer minimized motion but did not impede respiration. The design of the restrainer allowed adaptation to rats of differing sizes (Smith et al, 1985). The chamber was fixed to the restrainer and the window cleared with distilled water using a cotton wool stick. The restrainer was mounted on the vertical stage of the microscope with the rat in a ventral side down position. Video recordings with low magnification served to document the entire microvasculature and its angioarchitecture. In areas with optimal optical clarity, arteriolar and venular trees were selected. During a 3-minute period, recordings were made of consecutive segments of the vascular tree for off-line diameter measurements. The types of vessels were

grouped to the functional branching order and classifed alphanumerically. The primary perfusing arterioles of the muscle were designated as the first orders (A1) with their branches designated second orders (A2). Branches from the second and third order were third and fourth order (A3 and A4), respectively. The same vessel classification was used for the venous side (V1-V4). For arterioles with vasomotion (spontaneous rhythmic cycles of constriction and dilation) graphic recordings of arteriolar diameter were analyzed for maximal and minimal diameter, mean vessel diameter (maximal plus minimal/2), vasomotion amplitude (maximal minus minimal diameter/2), relative amplitude (ratio of vasomotion amplitude and mean vascular diameter) and frequency of vasomotion. The total observation period of each rat lasted 90 min. Central hemodynamic parameters (MAP, HR) were measured as explained in detail in chapter 2.

All data are presented as mean $\pm$ SEM. Data of SHR and WKY were compared, using Student's t-test for unpaired observations. Differences were considered statistically significant if $p<0.05$.

4.3 Results

All surgical procedures were successfully performed in 5 SHR and 7 WKY rats and fulfilled our criteria for physiological conditions. Results of central hemodynamic parameters are presented in table 4.1. The adult SHR showed a markedly higher blood pressure, with an average increase of 31 mm Hg. At this age (10-12 weeks), no differences in HR were found beween SHR and WKY at statistically significant levels.

The general network anatomy in both SHR and WKY were similar. Most arterioles formed non-symmetric bifurcating trees. Arteriolar arcades with multiple feeding arterioles (A1) were observed in 2 SHR and 3 WKY. Arcades at the arteriolar side were oberved for the A2 and A3 vessels with sometimes pendiculating flow patterns. Similar vascular arrangements were noticed at the venular side.

In fig. 4.1, mean arteriolar diameters for successive branching orders are presented. The magnitude of the differences between vessels in SHR and WKY varies with particular branching orders. A decreased

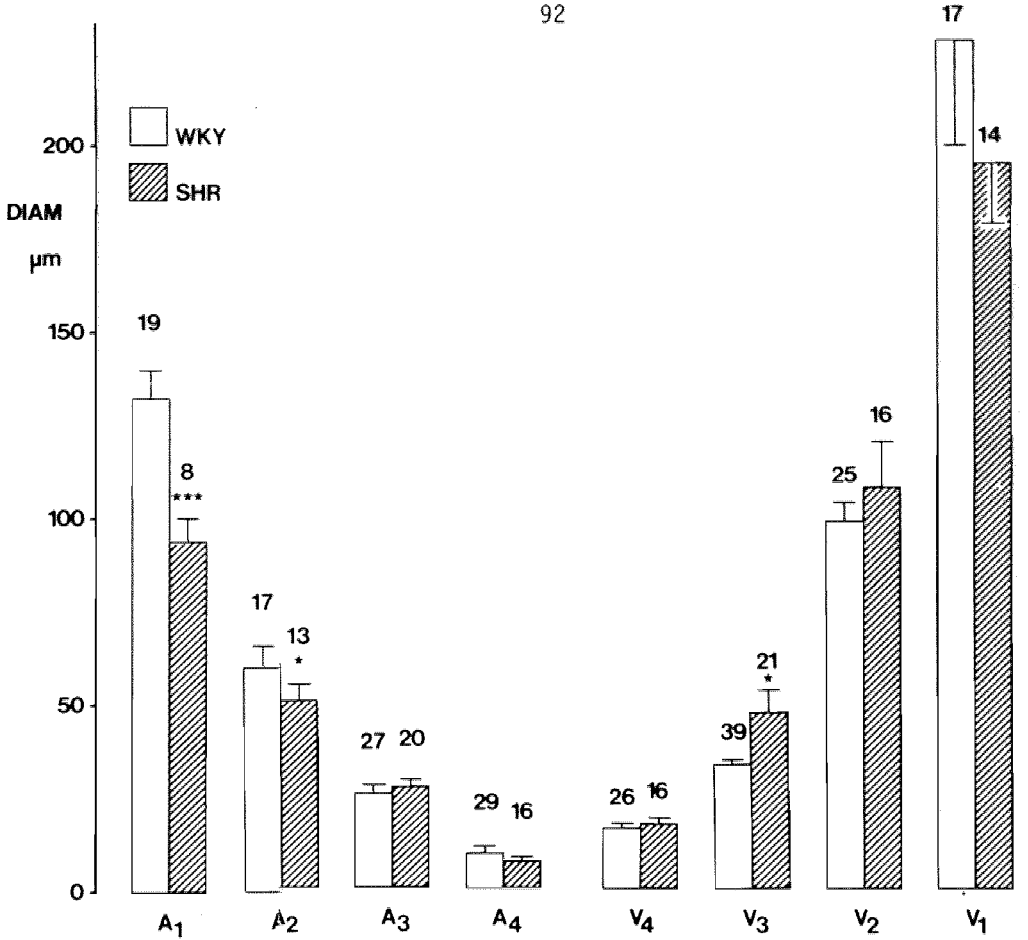

Fig. 4.1: Average diameter of each category of arteriolar and venular vessels. Data are expressed as mean ± SEM. Numbers above bars indicate number of vessels analyzed in 5 (SHR) or 7 (WKY) animals, and the asterisk denotes a statistical significant difference between SHR and WKY. (*p<005; ***p<0001).

Table 4.1: Central hemodynamic parameters (24-hour averages). Mean arterial pressure (MAP) and heart rate (HR). Data are presented as mean ± SEM.

	WKY	n	SHR	n	
MAP (mm Hg)	110±2	7	141±2	5	$p<0.001$
HR (bpm)	304±4	7	312±14	5	n.s.

lumen was found for first and second order arterioles in SHR. The most pronounced decrease (28%; $p<0.001$) was determined for the first order arterioles (SHR: 94.6±6.9 µm; WKY: 130.5 ±7.8 µm) while second order arterioles had diameters smaller than 21% as compared to WKY (SHR: 50.6±5.3 µm; WKY: 64.7±5.7 µm). Third and fourth order arterioles had equal diameters. In SHR, vessel diameters of postcapillary venules were marginally increased and only statistically different from control for the V3 vessels (V3 SHR: 47.9±6.7 µm; V3 WKY: 33.1 ±1.9 µm; $p<0.05$) as can be seen in fig. 4.1 as well. It should be noted that the largest venules (V1) in SHR had diameters slightly smaller than in WKY.

Restricted parts at the arteriolar side of the microvascular bed exhibited vasomotion. If vasomotion was present, it could only be observed in third and fourth order arterioles (table 4.2). Vasomotion was present in 30% of all SHR A3 vessels analyzed and in 41% of the A3 vessels in WKY. In SHR, 63% and in WKY 45% of all A4 vessels analyzed exhibited vasomotion. In most A4 vessels vasomotion showed an on-off behavior with periodic complete arteriolar closure and cessation of flow. Distally situated capillaries from these A4 vessels showed intermittent flow. A striking difference between SHR and WKY is an increased amplitude of vasomotion of A3 vessels in SHR. While mean calculated diameters were similar for this vessel category, the amplitude of vasomotion increased by 30% in SHR as compared to WKY (table 4.2).

Although A4 vessels in SHR had only slightly larger diameters (SHR: 6.9±0.7 vs. WKY: 5.4± 0.3 µm), it appeared to be statistically

different ($p<0.05$).

No differences in vasomotion frequencies were found between third and fourth order arterioles nor statistically significant differences between SHR and WKY. Only one A4 vessel in SHR did not exhibit a complete closure (relative amplitude 16.2%) while all A4 vessels in WKY showed the on-off phenomenon. The average vasomotion frequency varied between 6.0-6.8 cycles/min.

Table 4.2: Vasomotion parameters of third and fourth order arterioles.

	WKY	n	SHR	n	
Third order arterioles					
Mean diameter (µm)	22.4±2.8	11	20.3±1.2	6	n.s.
Frequency (cycles/min)	6.1±0.5	11	6.7±0.5	6	n.s.
Amplitude (µm)	6.4±0.7	11	10.7±2.2	6	$p<0.05$
Relative amplitude (% of mean)	30.6±3.2	11	60.3±11.7	6	$p<0.05$
Fourth order arterioles					
Mean diameter (µm)	5.4±0.3	13	6.9±0.7	10	$p<0.05$
Frequency (cycles/min)	6.0±0.4	13	6.8±0.5	10	n.s.
Amplitude (µm)	5.4±0.3	13	6.1±0.8	10	n.s.
Relative amplitude (% of mean)	100±0	13	91.6±8.0	10	n.s.

4.4 Discussion

4.4.1 Animal preparation

The preparation we used consisted of preformed tissue and has been preserved intact through careful surgical procedures. It appeared to be very important to use sterile surgical techniques and systemic antibiotic therapy. If surgery was properly performed rats showed a quick post-operative recovery, minimal weight loss and no signs of infections. During handling, all rats used in these experiments seemed to be comfortable and not limited in their normal behavior by the implanted chamber. The healing period of the chamber lasted for approximately 5-7 days and consisted of edema reabsorption. After a maximal healing period of 1 week, vasomotion in small arterioles was usually present. Trauma during surgery to the wall of blood vessels and microbleedings should be meticulously avoided because they prolong healing time. In addition, the presence of extravascular red blood cells and serum within the preparation can elicit angiogenesis (Hudlicka, 1984), thereby disturbing normal angioarchitecture of the muscle. It is hard to compare the muscle with the pre-operative state but the chamber was constructed to preserve the normal environmental conditions such as ion concentrations, gas tensions and temperature (Smith et al, 1985). Furthermore, vasomotion was present in our preparation and this can also be observed in tissues like the nail fold, the bat wing or dorsal skin fold of the hamster (Fagrell et al, 1980; Funk et al, 1983; Colantuoni et al, 1984). Deterioration of the preparation used in this study was usually manifest after 5-6 weeks. Deterioration can be observed as a development of granulation tissue. In that case, intravital microscopic observations are then also difficult because optical clarity is hampered and vasomotion is absent. However, in this study observations were done 2-3 weeks following implantation of the chamber. No signs of deterioration had occurred by that time.

4.4.2 Angioarchitecture

The arteriolar network of the cutaneous maximus muscle showed a large variety in topology. Arteriolar arcades were present for the A2

and A3 vessels. This indicates that the A1 and A2 vessels are responsible for local blood supply and distribution but that the regulation of blood flow and capillary perfusion is predominantly restricted to A3 vessels and transverse arterioles (A4 vessels). Experiments studying arteriolar recruitment following topical application of vasodilators are therefore necessary.

4.4.3 Diameter analysis

The increased resistance during the established phase of hypertension has been attributed to arteriolar constriction or structural factors related to an increased wall to lumen ratio due to encroachment on the lumen of a thickened vessel media (Folkow, 1983; Mulvany, 1978). The key finding of the comparison between microvascular diameters in skeletal muscle of conscious SHR and WKY in the present study is a decreased lumen for first and second order arterioles entering the muscle.

Several investigators studied microvascular diameters of skeletal muscle in adult rats. From these studies it seems that genetic hypertension is not associated with a general vasoconstriction of resistance vessels, sometimes even a dilatation was observed (Bohlen and Lobach, 1978; Zweifach et al, 1981; Henrich and Hertel, 1982). Only in one study, arteriolar constriction was observed in mature SHR (Prewitt et al, 1982). Definite conclusions cannot be drawn from these studies because they all used anesthetized animals. The importance of small arteries and arterioles in general to the increased peripheral resistance was recently discussed by Bohlen (1986). Small arteries are probably responsible for the bulk of the increased resistance and a small reduction in diameter accounts for a steep increase in resistance (Mayrovitz and Roy, 1983). This was demonstrated by micropressure measurements revealing a considerable and steep pressure drop of 40-60% already in primary arterioles entering the cremaster preparation (Bohlen et al, 1977). Apparently, a major fraction of vascular resistance lies proximal to the microvasculature in the feed vessels (Segal and Duling, 1986). A second method is the power dissipation measure which demonstrated the highest resistance to flow for larger arterioles (Borders and Granger, 1986). The reason of a greater con-

striction of larger arterioles may be a more dense innervation by the sympathetic nervous system (Marshall, 1982), an increased neurogenic tone in SHR (Judy et al, 1976) or alterations of the rheological properties of the arteriolar wall.

Resting lumen diameters of third and fourth order arterioles were not different from those found in WKY. Similar results were also obtained for anesthetized animals from the cremaster, gracilis and spinotrapezius muscle. This means that there is no increase in vascular resistance due to vasoconstriction of the terminal portion of the arteriolar bed. In SHR, the peripheral resistance is increased by approximately 50% (Bohlen, 1986) and small arterioles are responsible for 15% of the increased vascular resistance.

Thus, mechanisms other than reduced vascular lumen should be responsible. A reduced number of perfused arterioles has been proposed (Hutchins and Darnell, 1974; Chen et al, 1981; see also chapter 3). A second mechanism has been described recently. In the spinotrapezius muscle, the length ratio of arcading arterioles per volume tissue was increased without any evidence for anatomical rarefaction (Engelson et al, 1986). In our preparation, arteriolar arcades were observed. The present experiments were not designed to further study these possibilities.

The smaller vessel size of first and second order arterioles includes important consequences for the efficacy and physiological approach of pharmacological treatment of spontaneous hypertension (Zusman, 1986). A variety of vasodilators, nowadays frequently used in combination with diuretics or beta-blockers for long-term treatment of hypertension should primarily affect larger arterioles or small arteries.

Postcapillary venules in SHR (V2-V4) were dilated as compared to WKY. It is possible that postcapillary dilatation prevents an increased capillary filtration by changing the pre- to postcapillary resistance ratio. The basal mechanism(s) remain(s) to be explained. Diameters of larger venules (V1) were slightly less in SHR. Since the V1 vessels belong to the capacitance vessels a decreased diameter in SHR fits well with earlier observations revealing a decreased regional blood volume in spontaneous hypertension in rats (Evenwel et al,

1983).

4.4.4 Vasomotion

Only arterioles exhibited vasomotion and if present, in third and fourth order arterioles. Rhythmic diameter changes were not observed in first and second order vessels.

Few authors report about vasomotion in skeletal muscle arterioles in hypertension. Borders and Zweifach (1979) found a decrease in vasomotion amplitude and a concomitant increase in vascular resistance in rat spinotrapezius muscle. These rats were 7-9 weeks old and at this age, hypertension is characterized by overperfusion of the skeletal muscle vascular bed. Rats we used were in the early established phase with a still normal regional distribution of cardiac output but an already increased organ resistance (Smith et al, 1979; Evenwel et al, 1983; Iriuchijima, 1983). The increased vasomotion amplitude of A3 vessels in SHR in our study indicates a local reduction of vascular resistance. The cause of changes in vasomotion parameters in SHR remains unclear. In mature SHR, the same arterial inflow has to be distributed over the muscle by a lowered number of arterioles (Hutchins and Darnell, 1974; Chen et al, 1981; Prewitt et al, 1983). The increased amplitude of vasomotion may serve as a mechanism to restore local tissue blood flow and supply the capillaries.

In summary, the results support the hypothesis as advanced by Folkow (1983) that an increased peripheral resistance in SHR can be the result of a smaller arteriolar lumen of resistance vessels. In conscious rats, skeletal muscle arterioles, responsible for the bulk of the increased peripheral resistance were markedly narrowed in hypertension. More longitudinal studies in conscious animals are needed to elucidate the mechanism of microvascular network changes in SHR. The dorsal microcirculatory chamber technique used in this study provides an outstanding model to accomplish this goal.

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5. ADRENERGIC STIMULATION OF THE RAT MESENTERIC VASCULAR BED: A COMBINED MACRO- AND MICROCIRCULATORY STUDY

5.1 Introduction

The basic abnormality in chronic essential hypertension is an increased peripheral resistance. One of the primary factors controlling vascular resistance is the sympathetic nervous system. A large number of observations in various animal models of hypertension supports the concept that an increased sympathetic tone is a key event in the development of hypertension.

Vascular smooth muscle contraction by sympathetic stimulation is mediated primarily by the $alpha_1$-adrenoceptor subtype, whereas postjunctional $alpha_2$-adrenoceptors form the major target for circulating catecholamines (Timmermans and Van Zwieten, 1982; Alabastar and Davey, 1984). Exogenous application of adrenoceptor agonists is an often used method to mimick the effects of enhanced sympathetic activity. Thus, long-term elevation of circulating levels of noradrenaline by chronic intravenous noradrenaline infusion causes a sustained increase in vascular resistance and arterial pressure (Kleinjans et al, 1984). However, it has not yet been established whether activation of sympathetic nerves leads to similar effects on vascular resistance as the exogenous application of adrenoceptor agonists. In fact, both in vitro and in vivo data suggest that vasoconstrictors or neurogenic stimuli may produce similar increases in total resistance with strongly different effects on consecutive vascular segments (Nilsson, 1984; Grega and Adamski, 1987).

The purpose of the present study was to compare the effects of sympathetic nerve stimulation with those of exogenous application of adrenergic agents on microvascular dynamics. The mesenteric preparation was chosen as the object of study for several reasons: (1) the relatively easy access for intravital microscopic studies; (2) the dense sympathetic innervation of this tissue as judged by the marked reflex responses of the splanchnic bed to somatic and visceral stimuli (Gootman et al, 1983; Nijhof et al, 1983); (3) the splanchnic vascular resistance is among the first to rise in the spontaneous form of

hypertension in rats (SHR; Evenwel et al, 1983). The influence of endogenously released noradrenaline by sympathetic nerves was investigated by electrical stimulation of the posterior hypothalamus. Pharmacological stimulation was performed by using the natural neurotransmitter noradrenaline. Phenylephrine was chosen for its selective alpha$_1$-adrenoceptor agonist activity. A third vasopressor substance - angiotensine II - was chosen for its actions independent of alpha-adrenoceptor stimulation. Vasopressor substances were given via extravascular and different intravascular routes in order to study their basic actions with minimal interference of systemic reflex mechanisms.

5.2 Experimental protocol

Only WKY rats between 275-340 grams were used in this study. Figure 5.1 depicts the general lay-out of the experimental set-up. The different sets of experiments are summarized in table 5.1. It provides the route of drug administration, the different vasopressor substances used, the number of animals per group, and which techniques or methods were used in the different experiments. For an extensive description of all preparative techniques used the reader is referred to chapter 2. In general, experiments started 30-45 min after completion of all surgical procedures and equilibration of the preparation. In all sets of experiments, MAP and HR were measured throughout the experiment.

In the first set of experiments, vasopressor substances were given intravenously and each injection was preceded by a 3-minute period to obtain baseline values. When intravital microscopy of the mesentery was performed red blood cell velocity (section 2.10.2) and arteriolar diameter (section 2.10.1) were measured and flow through the superior mesenteric artery was measured using Doppler flowmetry (section 2.5.1). In the same animal, dose-response curves were made for NA, PE and AII, starting with the lowest dosages. Following injection, all hemodynamic variables were measured and recorded until the initial values were reached again. Between each injection, 10 min were allowed for recovery. In the second set of experiments, the same drug administration protocol was used, but now drugs were administered i.a.

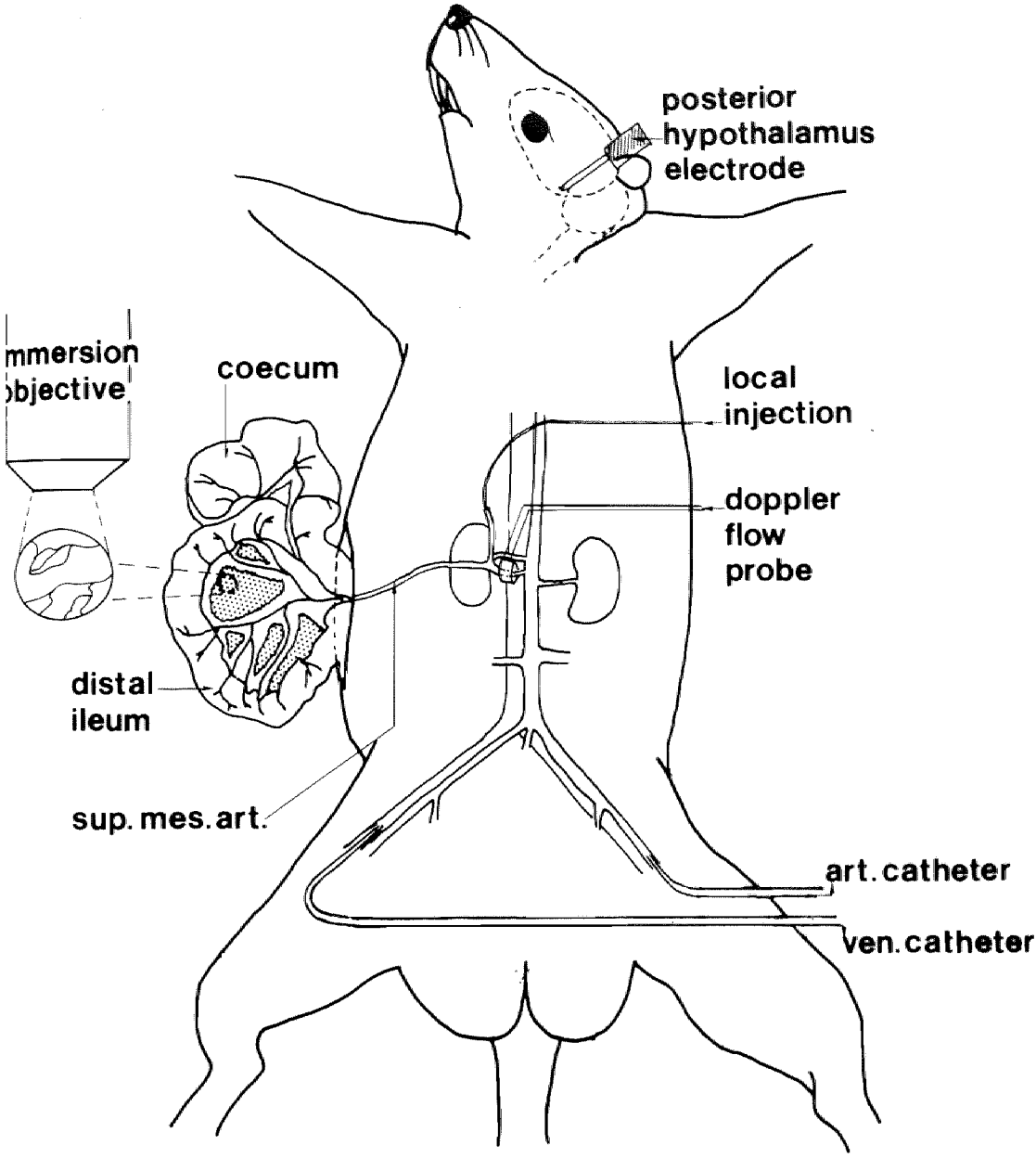

Fig. 5.1: General lay-out of the experimental set-up for the mesentery preparations and regional flow measurement.

Table 5.1: Different subsets of experiments in the present study.

Route of drug administration or stimulation	Drugs used	Methods		
		Intravital microscopy combined with Doppler flowmetry	Intra-vital microscopy	Doppler flowmetry
Intravenous	NA, PE, AII	n=4 v=4	n=6 v=6	n=8 -
Intra-arterial	NA	n=4 v=4	n=3 v=3	- -
Topical	NA	- -	n=8 v=10	- -
Posterior hypothalamus		- -	n=5 v=7	- -

n = number of animals, v = number of vessels, NA: noradrenaline, PE: phenylephrine, AII: angiotensin II.

instead of i.v. Drugs were administered locally to achieve higher concentrations at the receptor site of the vessels under study. Furthermore the involvement of systemic reflex effects can be minimized. In the third set of experiments, noradrenaline was directly superfused on the mesentery. During these experiments, inner arteriolar diameter was measured. When the maximum effect was reached the preparation was superfused with a fresh Tyrode's solution. Between each superfusion, 10 min were allowed for recovery. In the last set of experiments, only intravital microscopy was used and the microvascular effects upon hypothalamic stimulation were studied.

5.2.1 Drugs used

Three vasopressor substances were used: noradrenaline, phenylephrine and angiotensin II. Noradrenaline, the most important neuro-

transmitter, released upon sympathetic stimulation, stimulates post-synaptic alpha-adrenoceptors. Phenylephrine is a synthetically derived pressor agent and is more selective in stimulating the $alpha_1$-adrenoceptor subtype. Angiotensin II exerts its effect by a direct action on the vessel wall independent of alpha-adrenoceptor activation.

Noradrenaline ((-)arterenol bitartrate salt; NA), phenylephrine ((-)phenylephrine-hydrochloride; PE) and angiotensin II (Hypertensin; AII) were dissolved in saline (0.9% NaCl). Pressor agents were given as bolus injections either intravenously or intra-arterially (i.v. or i.a., respectively) via the femoral vein or the superior mesenteric artery in a volume of 50 µl. The following dosages were used in increasing concentration: noradrenaline 0.1-0.3-1.0-3.0 µg/kg; phenylephrine 1.0-3.0-10 µg/kg and angiotensin II 3-30-300 ng/kg.

In some experiments, noradrenaline was superfused on the mesentery (section 2.4.3). To this end, noradrenaline was dissolved in increments of a factor of 3 in a Tyrode solution in concentrations varying between 0.3-300 µg/l (10^{-9}-10^{-6} M). For each step, a maximal volume of 40-50 ml/min of the noradrenaline solution was applied topically by a Harvard infusion pump, within 3-5 min. The regular superfusion rate was 7-10 ml Tyrode's solution. All solutions were freshly prepared on the experimental day. Noradrenaline was protected from light by aluminum.

All parameters were recorded on a multichannel physiological recorder (Schwarzer).

The data are expressed as mean $\pm$ SEM. Changes in hemodynamic parameters are presented as percentage of control. The maximal effect on the parameters was used for further analysis.

5.3 Results

Base-line values of the different groups of experimental animals are summarized in table 5.2.

Table 5.2: Control values of mean arterial pressure (MAP), heart rate (HR), vessel diameter (Ø) and red blood cell (RBC) velocity in the diffent groups of animals used in this study (mean ± SE).

	MAP (mmHg)	HR (bpm)	Ø (µm)	RBC velocity (mm/s)
Intravenous injections				
Combined (Doppler and intravital microscopy)	116+4 (n=4)	262+11 (n=4)	20.5+2.9 (n=4)	2.6+1.5 (n=4)
Intravital microscopy	112+2 (n=6)	287+17 (n=6)	18.2+0.7 (n=6)	2.2+0.4 (n=6)
Doppler flowmetry	98+4 (n=8)	292+5 (n=8)	--	--
Intra-arterial injections				
Combined (Doppler and intravital microscopy)	122+6 (n=4)	310+15 (n=4)	23.0+3.9 (n=4)	3.2+0.3 (n=4)
Intravital microscopy	105+8 (n=3)	258+31 (n=3)	20.2+2.7 (n=3)	3.7+1.5 (n=3)
Topical application	122+3 (n=8)	302+11 (n=8)	22.3+2.3 (n=10)	--
Central neural stimulation	125+5 (n=5)	311+12 (n=5)	21.9+1.9 (n=7)	3.0+0.3 (n=7)

5.3.1 I.v. bolus injections

Fig. 5.2 presents a typical recording following the i.v. administration of 1.0 µg/kg noradrenaline. Blood pressure increased while blood flow through the superior mesenteric artery decreased. Maximal changes in these variables were usually observed within 5-10 s following injection.

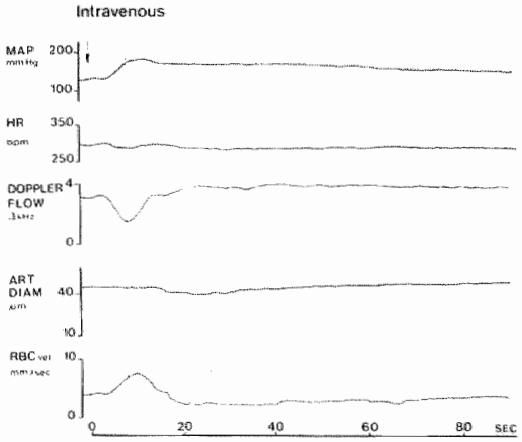

Fig. 5.2: Effects of an intravenous bolus of 1.0 µg/kg noradrenaline on mean arterial pressure (MAP), heart rate (HR), blood flow through the superior mesenteric artery, mesenteric arteriolar diameter and red blood cell velocity (RBC vel). The injection was given at t=0. The dotted line marks the moment of maximum change in organ blood flow.

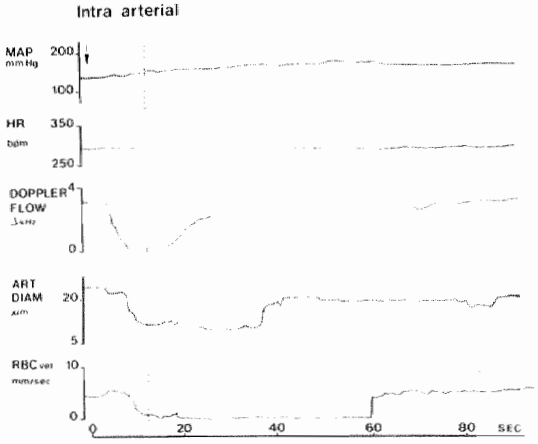

Fig. 5.4: Effects of an intra-arterial bolus of 1.0 µg/kg noradrenaline. Mean arterial blood pressure (MAP), heart rate (HR), regional blood flow (superior mesenteric artery; Doppler flow), mesenteric arteriolar diameter (art diam) and red blood cell velocity (RBCvel). The injection was given at t=0. The dotted line marks the moment of maximum change in organ blood flow.

The mesenteric arterioles showed the following response. At the moment that MAP increased, red blood cell velocity tended to increase, reached its maximum at 10-15 s and then gradually declined. Vascular constriction could only be observed between 8-15 seconds after the maximal increase in blood pressure. This was consistently found in all experiments. Similar results were obtained after i.v. administration of phenylephrine and angiotensin II. Bolus injections of only saline did not cause any effect.

The maximum increase in blood pressure was 64.7±5.7%, 48±3.9% and 45.7±2.8% for the highest dosages for respectively NA, P and AII. Higher dosages were not used because of severe side-effects like arrhythmias. Fig. 5.3 summarizes all data with the exception of HR, since this variable did not change significantly in any of the experimental groups.

5.3.2 I.a. bolus injections

A series of experiments were performed to study local vascular reactivity while minimizing systemic effects by injecting vasopressor substances directly into the superior mesenteric artery. Fig. 5.4 presents a typical recording after i.a. injection of 1.0 µg/kg noradrenaline. Mesenteric artery blood flow decreased sharply and was followed by a decrease in arteriolar diameter and arteriolar red blood cell velocity. The decrease of regional blood flow ran parallel to changes in arteriolar diameter. Administration of the vehicle solution did not have any effect. Table 5.3 compares the maximum changes in hemodynamic variables obtained with different doses of i.a. versus i.v. noradrenaline obtained in the animals in which combined Doppler and intravital microscopic measurements were made. The data show that, in contrast to i.v. administration, i.a. injection of noradrenaline always leads to a reduction of RBC vel, even at the low doses of 0.01 and 0.03 µg/kg which do not affect MAP. Furthermore, the data show a much stronger effect of i.a. noradrenaline on arteriolar diameters. At the two highest doses there is almost complete arteriolar closure and stasis of the RBCs.

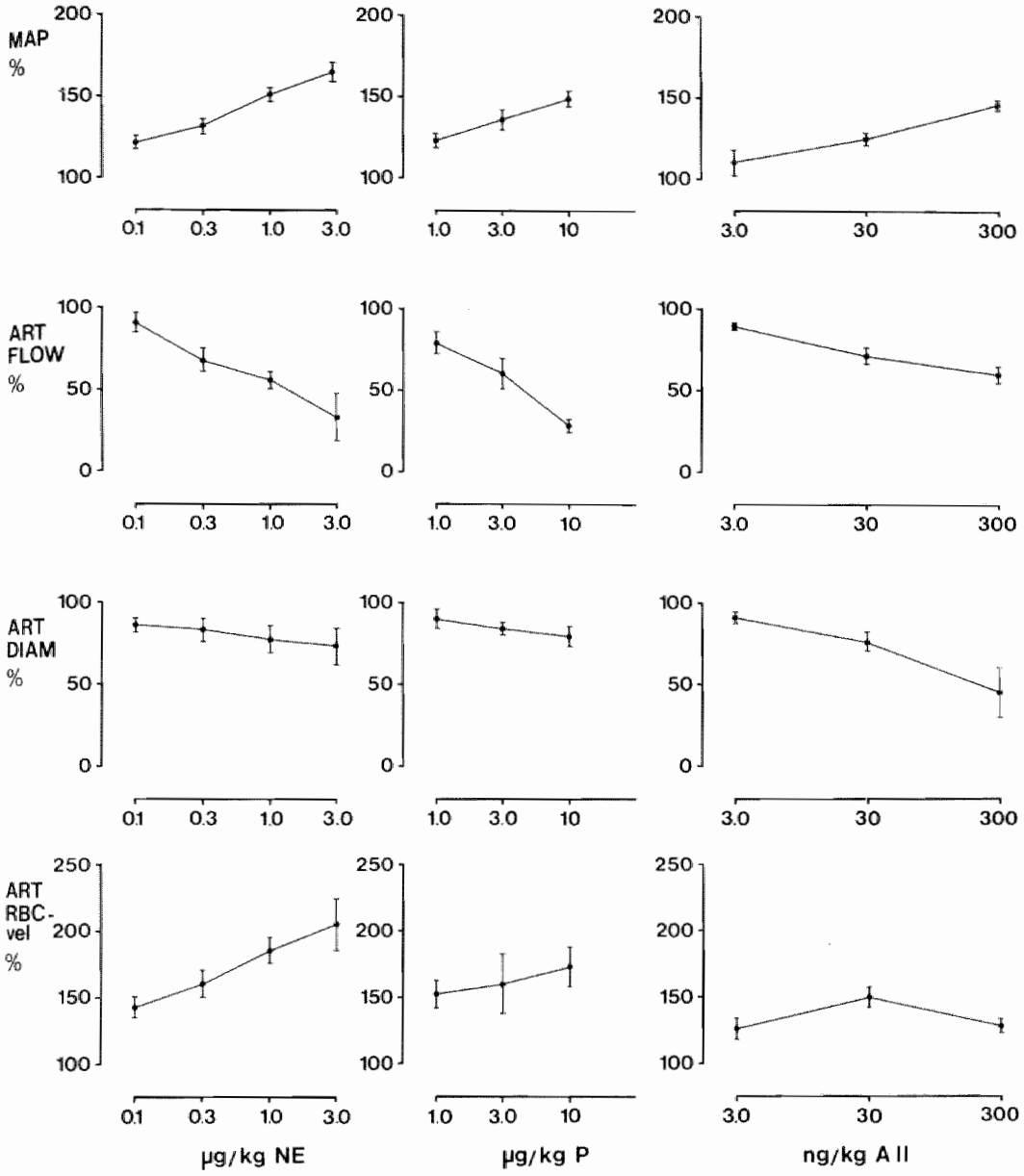

Fig. 5.3: Effects of different intravenous doses of noradrenaline (NE), phenylephrine (P) and angiotensin II (AII) on mean arterial pressure (MAP), superior mesenteric artery blood flow, mesenteric arteriolar diameter and red blood cell velocity (RBC vel).

Table 5.3: Changes in mean arterial pressure (MAP), Doppler blood flow, arteriolar diamter (Ø) and red blood cell velocity (RBC vel) during intravenous or intra-arterial administration of different doses of noradrenaline in the rat (mean ± SE; n=4)

	MAP (%)		flow (%)		Ø (%)		RBC vel (%)	
Dose (µg/kg)	i.v.	i.a.	i.v.	i.a.	i.v.	i.a.	i.v.	i.a.
0.01		+1±1		-12±4		-18±5		-31±11
0.03		+4±1		-23±6		-22±6		-50±18
0.1	+27±5	+11±3	-18±4	-46±8	-12±5	-40±7	+44±12	-70±15
0.3	+43±5	+20±5	-41±12	-70±8	-15±4	-72±9	+62±11	stasis
1	+79±21	+41±7	-49±9	-91±10	-24±10	-95±7	+85±14	stasis
3	+78±7		-69±6		-30±13		+77±20	

5.3.3 Topical administration of noradrenaline

Topical administration of noradrenaline consistently caused an arteriolar constriction. The maximum effect on arteriolar diameter was noticed when 10^{-6}M noradrenaline was applied. Solutions with a higher concentration provoked irreversible effects like stasis which did not disappear after superfusion with fresh Tyrode's. Although arteriolar red blood cell velocity was not measured consistently in these experiments, an increase in RBC velocity was never observed. The results are summarized in fig. 5.5.

5.3.4 Central neural stimulation

Stimulation of the posterior hypothalamus was successfully performed in 5 animals. A total number of 7 A_2 vessels were studied. Fig. 5.6 shows an original recording with stimulation of 64 Hz. Blood pressure (+42±3%; n=5) and HR (+19±6%; n=5) increased as a result of the sympathetic stimulation. A considerable delay (10-30 s) between the effects on blood pressure and arteriolar constriction was observed as was also found after the i.v. bolus injections. The maximum decrease in diameter amounted to 40±13%. Red blood cell velocity in-

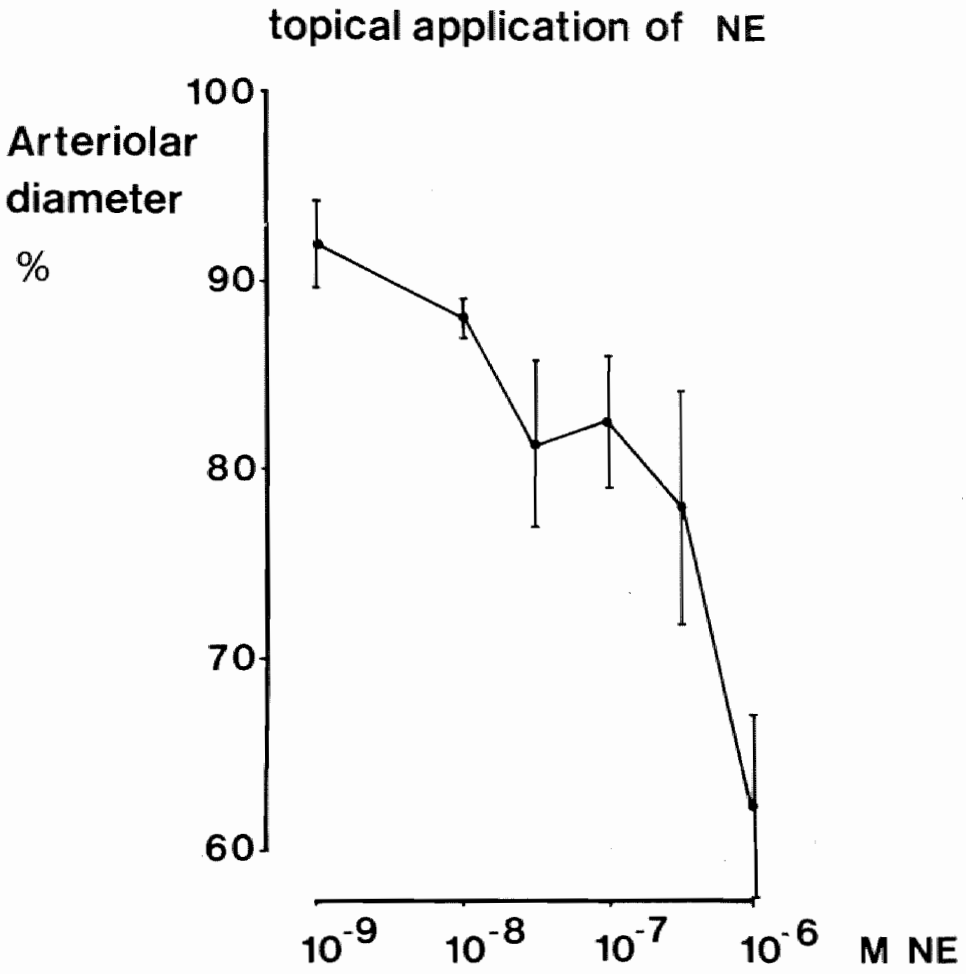

Fig. 5.5: Effects of topical administration of different concentrations of noradrenaline (NE) on mesenteric arteriolar diameter.

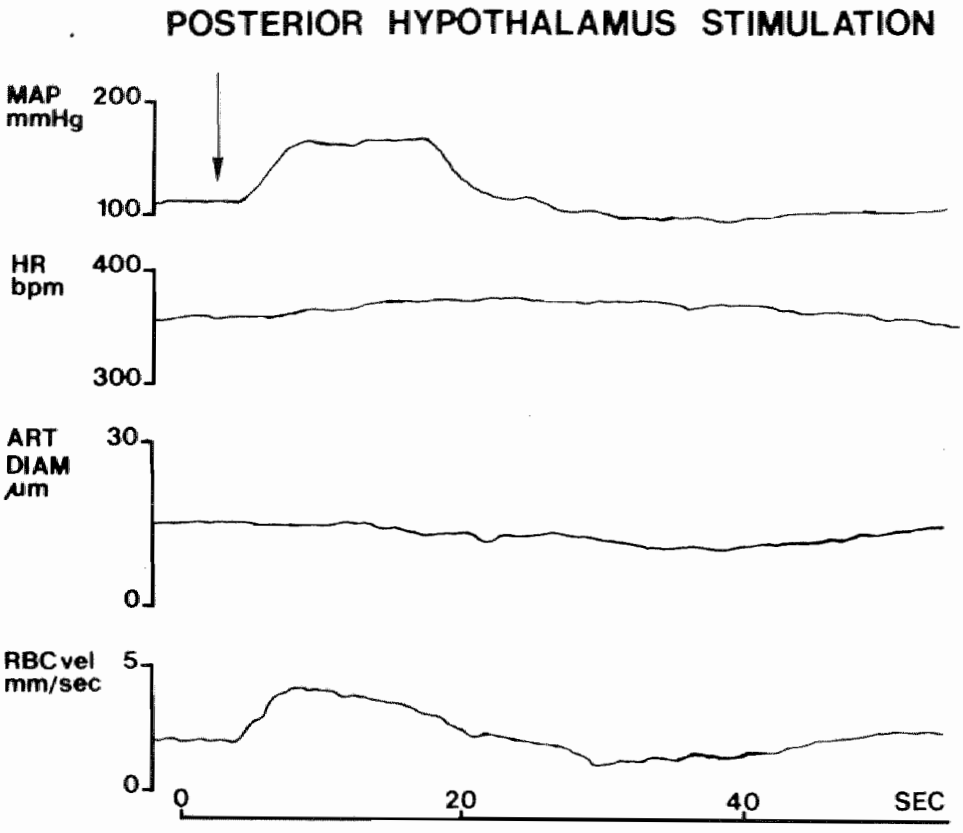

Fig. 5.6: Effects of electrical stimulation of the posterior hypothalamus on mean arterial blood pressure (MAP), heart rate (HR), arteriolar diameter (art diam) and red blood cell velocity (RBCvel). Stimulation was started at the point of the arrow and was continued for 15 s.

creased (+37±8%) in parallel to blood pressure increase.

5.4 Discussion

In this study, we demonstrated that exogenous application of (adrenergic) vasoconstrictors or neurogenic stimuli may produce an overall increase in total resistance to blood flow, with strongly different effects on consecutive segments within one vascular bed. Evidence for such divergent effects was thus far obtained in in-vitro, isolated ring preparations of arteries of different sizes (Nilsson, 1984) or in in-vivo, isolated perfused organ preparations (Grega and Adamski, 1987). In this study we have introduced a new in-vivo approach for combined macro- and microcirculatory studies in the intact rat. It combines the use of miniaturized Doppler flow probes for the continuous measurement of arterial blood flow with the intravital microscopy approach to study microcirculatory dynamics. The method is still based upon the use of anesthetized animals. In a previous study (Le Noble et al, 1984) we showed that for the study of the splanchnic vascular bed, pentobarbital is the anesthetic of choice, since it does not affect vascular sensitivity to pressor stimuli in this vascular bed significantly. Our intra-arterial and topical noradrenaline administration data confirm a good sensitivity of our preparation to a pressor stimulus. Noradrenaline produces a local vasoconstriction in doses well below those causing systemic effects.

A major result of our study was the difference in effects obtained with local (e.g. intra-arterial, topical) noradrenaline administration on the one hand and intravenous administration of this and other pressor agents as well posterior hypothalamic stimulation on the other hand. One could argue that these differences are related to divergent local drug concentrations with different routes of administration. It is technically hard, if not impossible, to measure the actual local drug concentrations in our experimental set-up. However, we can estimate possible differences in local concentrations on the basis of previously published pharmacokinetic models of local versus systemic

drug administration (Chen and Gross, 1979; Daemen et al, 1986). According to these models, the following relationships can be used:

$$\frac{C_{Tia}}{C_{Tiv}} = 1 + \frac{Cl_S}{Q_T}$$

in which C_{Tia} and C_{Tiv} are the drug concentration in the arterial blood in the target organ upon respectively intra-arterial and intravenous drug administration; Cl_S is the systemic drug clearance and Q_T is the organ blood flow. On the basis of published data on noradrenaline clearance (approximately 50 ml/min; Benedict et al, 1978; Esler et al, 1979) and rat splanchnic blood flow (approximately 10 ml/min; Evenwel et al, 1983), the quotient C_{Tia}/C_{Tiv} can be estimated to be in the range of 5-10.

In our experiment we have used a range of different concentrations of noradrenaline (i.a.: 0.01-1 µg/kg; i.v.: 0.1-3 µg/kg). This implies that we covered potential differences in local concentrations of a range exceeding the factor 5-10 calculated above. Even with this large range of obtained local concentrations we found both qualitative and quantitative differences with the two routes of administration. Intraarterial noradrenaline caused a decrease in arteriolar diameter, RBC velocity and Doppler flow, both at concentrations that did not influence MAP and that increased MAP. This implies a local vasoconstriction also at the level of the microcirculation. Intravenous noradrenaline, on the other hand, caused a decrease in Doppler flow, hardly affected arteriolar diameter and increased RBC velocity.

The increase in RBC velocity during intravenous noradrenaline administration may be caused by a redistribution or shunting of blood. The effect was consistently found for all three vasopressor substances, indicating a response independent of alpha-adrenoceptor stimulation, but rather linked to hemodynamic changes at a more central level, e.g. the increase in blood pressure. Banks et al (1985) have recently shown that splanchnic vasoconstriction is disproportionally reflected in the mucosa and submucosa. The muscularis seems to

be least reactive with regard to sympathetic stimulation. The bulk of blood flow through the superior mesenteric artery goes to the intestines and the mucosa and submucosa receive approximately 70% of this flow during resting conditions. The physiological function of resistance changes in the mucosa and submucasa may be a contribution to constant pressure and flow conditions essential for mucosal oxygen supply and intestinal absorption processes (Gore and Bohlen, 1977; Banks et al, 1985). The mesentery receives about 1% of total splanchnic flow (Richardson and Johnson, 1970) and can therefore play only a limited hemodynamic role. Furthermore, it belongs to the low priority vascular beds. Its possible role could be shunting of blood during acute changes in blood pressure in concordance with the muscularis layer and in this way it could contribute to the integrity of the vital parts of the intestinal tract. This study does not allow any conclusions as to the nature of the factors determining the redistribution of flow. However, the local reactivity data clearly show that the microvascular effects are not the result of a direct local action of the sympathetic stimuli via alpha-adrenoceptor stimulation.

Central nervous system stimulation is a generally accepted method to increase peripheral sympathetic tone to precapillary vessels (Ninomiya et al, 1970; Baez et al, 1977; Stoddard-Apter et al, 1983). Furthermore, chronic electrical stimulation of the posterior hypothalamus has been used to simulate the development of essential hypertension (Folkow and Rubinstein, 1966; Takeda and Bùnag, 1978). Rat mesenteric arterioles show an intense autofluorescence after pretreatment with formaldehyde, indicating a dense sympathetic innervation (Hertel and Henrich, 1981). In our study, electrical stimulation of the posterior hypothalamus increased blood pressure considerably. This area is particularly sensitive since no blood pressure increase was observed in animals with electrodes implanted just outside this area.

The hemodynamic pattern following hypothalamic stimulation was an increase in MAP with a constrictor response of mesenteric microvessels. The microvascular response did not run in parallel with the blood pressure increase. Basically, similar results were obtained following i.v. bolus injections of noradrenaline, suggesting a redistribution of flow, rather than a direct vasoconstriction. Baez et al

(1977) observed a microvascular constriction following stimulation of different sites in the forebrain and midbrain. However, it is difficult to compare their results with ours, since they stimulated different sites in the central nervous system, did not measure red blood cell velocity and did not report on the exact time course between changes in MAP and microcirculatory variables.

In conclusion, our results indicate that exogenous application of adrenergic and other vasoconstrictors or neurogenic stimuli may produce an overall increase in total resistance to blood flow in the splanchnic bed of intact rats, with different effects on the consecutive segments in this bed. The hemodynamic effects depend upon the way of stimulation or the route of drug administration. Interpretation of experimental results on the vascular reactivity of mesenteric microvessels should take into account the experimental design.

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6. DIFFERENTIAL EFFECTS OF GENERAL ANESTHETICS ON REGIONAL VASOCONSTRICTOR RESPONSES IN THE RAT

6.1 Introduction

Although there is a tendency towards the use of unanesthetized animals most microvascular studies require general anesthetics to externalize tissues for microscopic observations. It is well established that anesthetics interfere with cardiovascular reflexes like baroreflexes (Cox and Bagshaw, 1979) and chemoreflexes (Zimpfer et al, 1981). It has also been shown that anesthesia may affect the vasculature directly, thus influencing constriction or dilatation (Altura et al, 1980). In fact, urethane has been shown to interfere with alpha$_2$-adrenoceptor mediated vasoconstriction (Armstrong et al, 1982; Moore et al, 1984). These effects seem to depend upon interference of anesthetics with transmembrane calcium fluxes or mobilization of intracellular calcium (Altura et al, 1979; Altura et al, 1980; Maggi et al, 1984).

Effects on calcium metabolism may result in differential effects on vasoconstriction, depending upon the nature of the vasoconstrictor drug's cellular action as well as possible regional differences in the body in the way vasoconstriction may be achieved. Therefore, in the present study the effects of an alpha$_1$-adrenoceptor agonist (phenylephrine), a mixed alpha$_1$- and alpha$_2$-agonist (noradrenaline) and the non-adrenergic vasoconstrictor agent angiotensin II on regional hemodynamics in conscious rats and in rats anesthetized with pentobarbital or an alpha-chloralose/urethane mixture were compared. These anesthetics or combination of anesthetics were chosen based on their frequent use in microvascular studies. The regional responses were studied since they reflect arteriolar alterations.

6.2 Experimental protocol

Rats, weighing 275-300 g, were divided into 3 groups. Doppler

flow probes and catheters were implanted as described in chapter 2.

In group I (7 animals), surgery was performed under pentobarbital (PB) anesthesia (60 mg/kg i.p.). Following surgery, animals were allowed 3-4 days to recover and they were used in the conscious state.

In groups II and III, surgery was performed immediately prior to the experiments. In group II (8 animals), anesthesia was induced by slow injection of 30 mg/kg pentobarbital through a tail vein. In group III (10 animals), alpha-chloralose and urethane (C-U; 1-13.3%) were given in a dose of 3-4 ml/kg intraperitoneally. Prior to surgery, comparable anesthetic depth was evaluated by tail pinch. If necessary, additional anesthetic was given.

In groups II and III, the trachea of all animals was intubated to facilitate spontaneous breathing. Furthermore, a continuous infusion of 0.9% NaCl was given through the arterial catheter at a rate of 3 ml/hr to prevent dehydration (Maddox et al, 1977).

6.2.1 Drugs

Noradrenaline-bitartrate, phenylephrine hydrochloride, angiotensin II, and alpha-chloralose were purchased from Sigma (St. Louis, Mo). All substances were dissolved in 0.9% NaCl and solutions were freshly prepared immediately prior to the experiments.

Dose-response curves for noradrenaline (0.1-3 µg/kg), phenylephrine (1-10 µg/kg), and angiotensin II (3-300 ng/kg) were obtained in all animals. The order in which drugs were given as well as the doses were randomized.

After stabilization of hemodynamics, base-line values for mean arterial pressure (MAP), heart rate (HR) and flows were obtained as the means of 5 consecutive readings at 1 min intervals just before injections. Then, i.v. injections of vasopressor substances were given in a 50 µl volume. Between injections, 10-15 min were allowed for recovery of hemodynamics.

Blood flows were obtained as KHz Doppler shift. Effects are presented as percentage changes from control values. Resistance changes in the vascular beds were calculated from changes in MAP and changes in respective Doppler shifts and are expressed as percentage changes. A maximal increase of 500% for resistance was assumed if blood flow

approached zero.

All data are presented as mean ± SEM, unless indicated otherwise. Base-line values for MAP and HR in the 3 groups were compared with a one-way analysis of variance and a modified t-test (section 2.11). Dose-response curves were compared with one-way analysis of variance as described by Zerbe (section 2.11). Significance was defined at the 5% level.

6.3 Results

Base-line values for MAP and HR in the 3 experimental groups are summarized in table 6.1. As compared to conscious rats, pentobarbital anesthetized rats had a reduced HR, whereas MAP was not significantly different from that in conscious rats. In contrast, in chloralose/urethane anesthetized rats, MAP was reduced, but HR was similar to that in conscious rats.

Table 6.1: Baseline values for mean arterial pressure and heart rate (**p<0.01; **p<0.001).

	N	MAP	HR
Conscious	7	101±4	358±15
C-U anesthetized	10	83 ±4**	349±6
PB anesthetized	8	98 ±4	292±5***

6.3.1 Effects of noradrenaline (NA) in conscious and anesthetized rats

In all 3 groups of rats, NA increased MAP dose-dependently (cf. fig. 6.1A). The pressor response in PB-anesthetized animals was greater than that in conscious animals (p<0.01). C-U anesthesia did not significantly alter the pressor response. The reduction in HR that was observed following NA in conscious rats was significantly suppressed

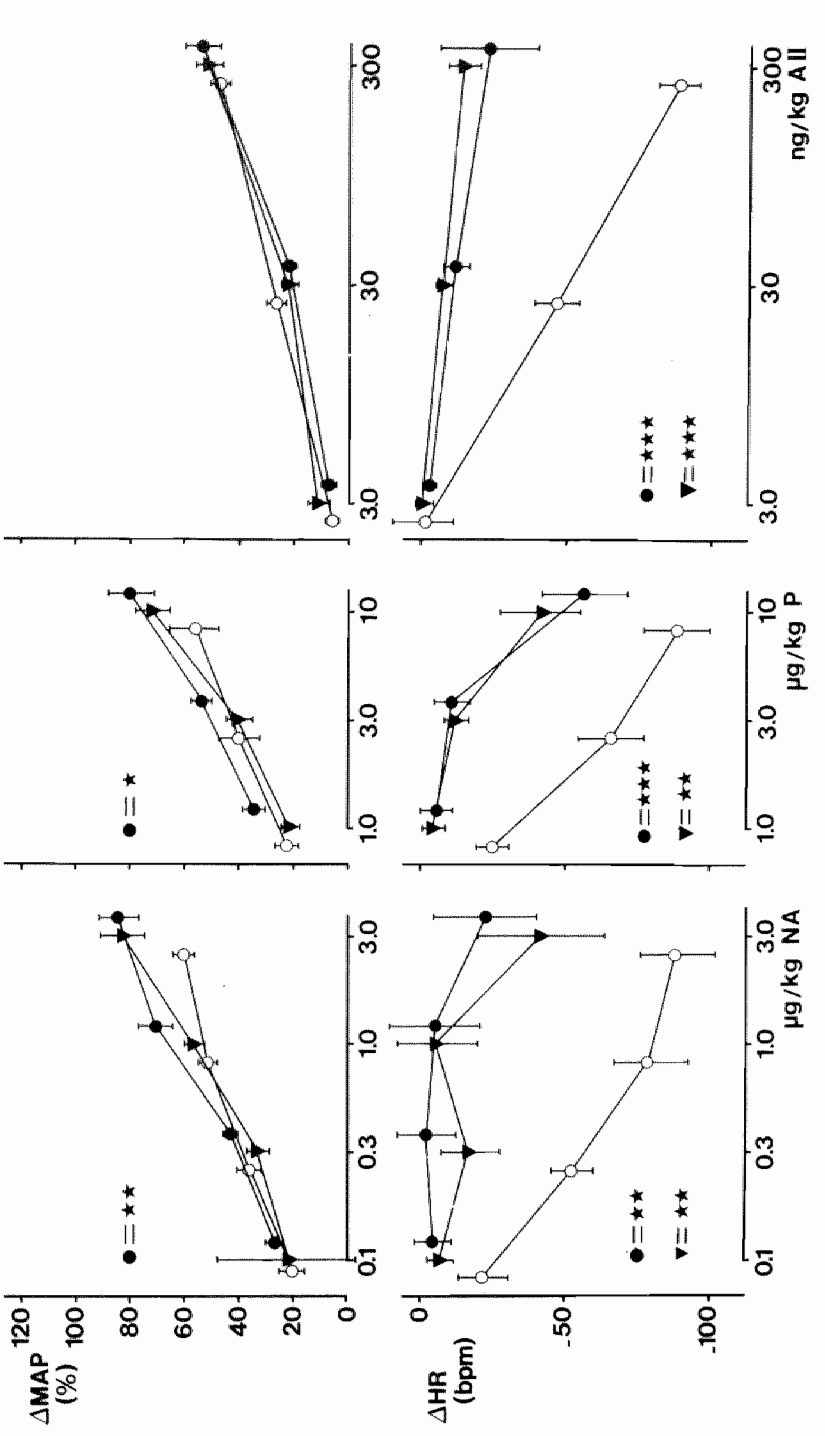

Fig. 6.1: Effects of noradrenaline (NA; 1A), phenylephrine (P; 1B), and angiotensin II (AII; 1C) on mean arterial pressure and heart rate in conscious (o - o), pentobarbital (● - ●), and chloralose-urethane anesthetized animals (▼ - ▼). For significances of the differences between conscious and anesthetized animals (*$p<0.05$; **$p<0.01$; ***$p<0.001$), cf. section RESULTS.

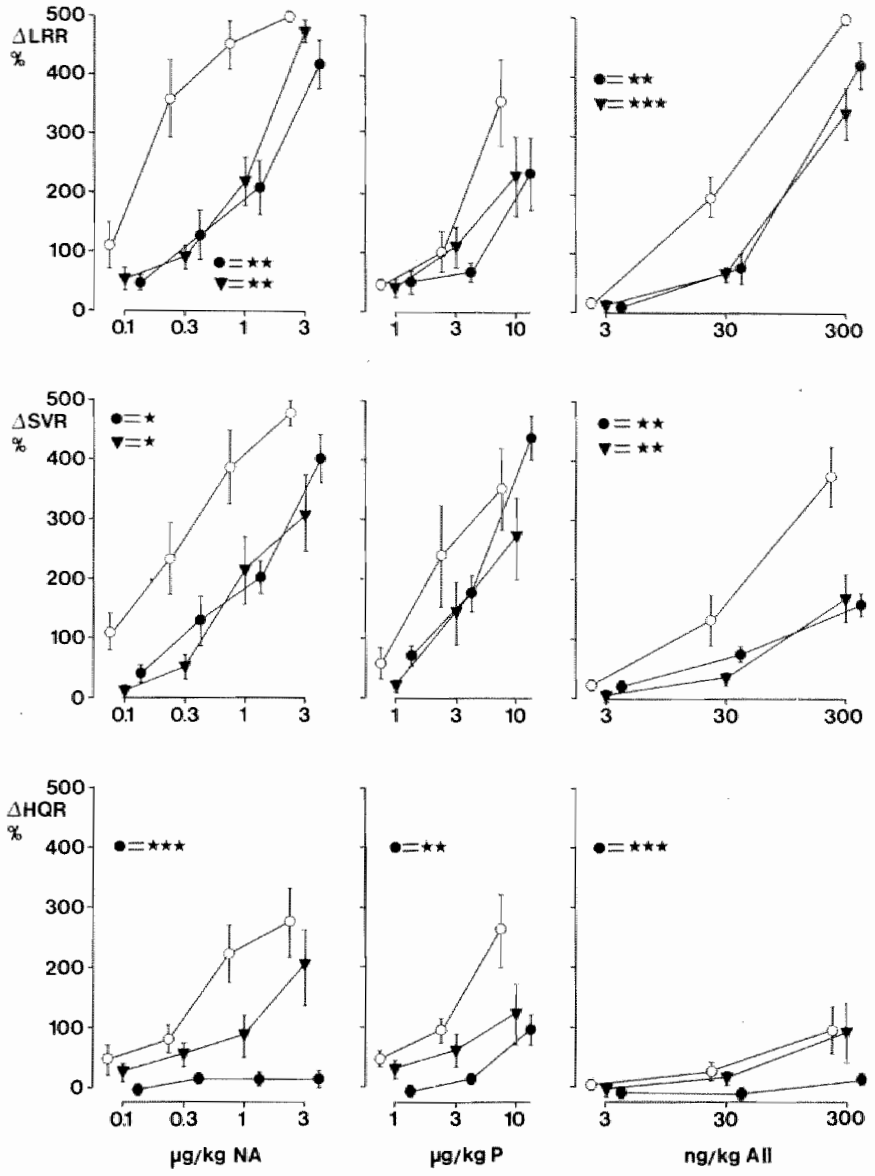

Fig. 6.2 Effects of noradrenaline (NA; 1A), phenylephrine (P; 1B), and angiotensin II (AII; 1C) on left renal resistance (LRR), splanchnic vascular resistance (SVR) and hindquarter resistance (HQR) in conscious (o - o), pentobarbital (● - ●), and chloralose-urethane anesthetized animals (▼- ▼). For significances of the differences between conscious and anesthetized animals (*p<0.05; **p<0.01; ***p<0.001), cf. section RESULTS.

in both PB-anesthetized ($p<0.01$) and C-U anesthetized animals ($p<0.01$).

Effects of anesthetics on NA-induced changes in regional vascular resistances are summarized in fig. 6.2A. In all 3 vascular beds, NA increased vascular resistance dose-dependently. In the hindquarters, this effect was smaller than in the kidney and splanchnic vascular bed (cf. fig. 6.2A).

Pentobarbital caused a general reduction of the vascular responses. In all 3 groups, vasoconstriction was inhibited. In the hindquarters, this resulted in complete abolition of vasoconstriction.

C-U had a differential effect on NA-induced vasoconstriction. Significant depression of the dose-response curve was noted in the renal ($p<0.01$) and splanchnic vascular beds ($p<0.05$), whereas vasoconstriction in the hindquarters was affected insignificantly. Differences between PB and C-U anesthetized animals were significant ($p<0.05$) only in the hindquarters.

6.3.2 Effects of phenylephrine (P) in conscious and anesthetized rats

P caused a dose-dependent increase in MAP in all 3 groups (fig. 6.1 B). The effect in PB-anesthetized animals was greater ($p<0.05$) than that in conscious rats. HR was reduced in all groups, but anesthesia inhibited this effect significantly (PB anesthesia: $p<0.001$; C-U anesthesia: $p<0.01$).

Regional resistances increased dose-dependently in all 3 groups of animals (cf. fig. 6.2B). Neither PB nor C-U significantly affected these vasoconstrictions, although there was a tendency towards a general depression.

6.3.3 Effects of angiotensin II (AII) in conscious and anesthetized rats

AII increased MAP dose-dependently and to a similar degree in all 3 groups of animals. As with the other vasopressor substances, HR decreased, but the bradycardia was significantly less in both C-U-anesthetized ($p<0.001$) and PB-anesthetized ($p<0.001$) animals than in conscious animals (cf. fig. 6.1C).

In conscious animals, AII caused general vasoconstriction (cf.

fig. 6.2C). The effect was least pronounced in the hindquarter vascular bed. PB anesthesia inhibited vasoconstriction in all 3 beds ($p< 0.01$). In contrast, C-U did not alter hindquarter vascular responsiveness, but did diminish the effects on renal ($p<0.001$) and splanchnic ($p<0.01$) vascular resistances.

6.4 Discussion

In the present study, we compared the effects of 3 different vasoconstrictor substances on regional hemodynamics in conscious, PB-anesthetized rats and C-U-anesthetized rats. Our choice of vasoconstrictor agents was related to their different modes of cellular action. Thus, phenylephrine has predominantly $alpha_1$-adrenoceptor action. Norepinephrine stimulates both $alpha_1$- and $alpha_2$-adrenoceptors, whereas angiotensin II predominantly acts through stimulation of specific angiotensin receptors. The choice of anesthetics was based upon their frequent use in cardiovascular research. The particular ratio of alpha-chloralose and urethane in the mixture was chosen on the basis of a study in rats (DeLano and Zweifach, 1981), comparing microvascular responsiveness under different anesthetic conditions. The ratio of 1:13.3 was proven there to cause least depression of the responsiveness.

Reports on the effects of pentobarbital and chloralose-urethane combinations on resting MAP and HR are conflicting. Thus, PB has been reported to lower MAP (Smith and Hutchins, 1980; Linas et al, 1980; Walker et al, 1983; Kawaue and Iriuchijima, 1984) or not to change MAP (Lee et al, 1985). Also chloralose-urethane combinations reduced (Smith and Hutchins, 1980) or did not affect MAP (DeLano and Zweifach, 1981). Effects on HR were similarly variable. Differences seem to depend upon doses of anesthetics, strain differences and, in the case of C-U, the ratio of the anesthetics within the mixture. In the present study, we observed a lowering of MAP but not HR by C-U, and reduced HR and unchanged MAP following PB. The doses were chosen on the basis of comparable depth of anesthesia.

All 3 vasoconstrictor agents caused dose-dependent increases in

MAP in conscious and anesthetized rats. PB tended to potentiate this response following norepinephrine and phenylephrine. Possibly this results from depression of baroreflexes that tend to correct blood pressure increases. Such depression by general anesthetics has been described (Cox and Bagshaw, 1979) and is evidenced in the present study by diminution of reflex bradycardia. However, if this were the only mechanism involved in the potentiation of responses on MAP, it should be expected that effects of all 3 vasoconstrictors were potentiated similarly by both anesthetics, because in all cases, there is comparable inhibition of reflex bradycardia. The reason for the differences between the different agents is not understood, but may depend upon venous actions.

Several studies have indicated that anesthetics influence vascular responsivenss to constrictor agents. In isolated rat aortae and portal veins, Altura and Weinberger (1979) found a decreased response to both adrenaline and angiotensin II following addition of urethane. In a study of isolated rabbit ear arteries, others also noted a reduction of noradrenaline-induced contractions by urethane (Maggi et al, 1984). Also, in microcirculatory studies of the cremaster muscle in rats, noradrenaline effects were inhibited by urethane (Miller and Wiegman, 1977). None of these studies did, however, address the possibility of regional differences in the effects. In one other study, vascular responses to hemorrhage during different types of anesthesia were studied (Seyde et al, 1985). These authors noted that especially the increases in muscle vascular resistance were inhibited by the use of either PB or urethane as compared to conscious animals. Of course, a major difference between their study and ours is the fact that in our preparation we did not primarily reduce cardiac output and effects did not depend upon reflexes.

In the present study, vasoconstriction occurred in all 3 vascular beds studied, following administration of noradrenaline, phenylephrine and angiotensin II in conscious rats. The magnitude of these responses diminished from renal > splanchnic > hindquarter. Thus, it would seem that the hindquarter vascular bed is a relatively passive bed, reacting to changes in MAP, which may be regared as autoregulatory responses.

Responses to the vasoconstrictor agents were influenced differentially both with regard to the anesthetic and to the vascular bed. Responses to phenylephrine in all 3 beds were not altered significantly. Effects to NA as well as AII were significantly diminished in all 3 vascular beds by PB anesthesia. And although C-U had similar inhibitory effects in the kidney and splanchnic vascular bed, C-U did not significantly alter responses to noradrenaline and angiotensin II in the hindquarter vascular bed.

General anesthetics have been shown to interfere with cellular calcium metabolism (review: Altura et al, 1980). Although both pentobarbital and urethane interfere with intra- as well as extracellular calcium related effects, the relative importance of the effects on the two mechanisms is unclear.

The cellular mechanisms resulting in vasoconstriction following $alpha_1$-adrenoceptor, $alpha_2$-adrenoceptor and angiotensin II receptor stimulation differ. Thus, the constrictor response to $alpha_1$ stimulation predominantly depends upon a mobilization of intracellular calcium, whereas postsynaptic $alpha_2$-adrenoceptor stimulation results in an influx of calcium into the cell (Van Meel et al, 1981). Angiotensin II-induced vasoconstriction depends upon facilitation of calcium influx and mobilization of intracellular calcium (Deth and Van Breemen, 1977). On this basis, phenylephrine, being an $alpha_1$-adrenoceptor agonist, should be expected to behave differently from the mixed $alpha_1$- and $alpha_2$-agonist noradrenaline as well as angiotensin II. The latter two should behave similarly.

From the present study, where we found that the effects of $alpha_1$-adrenoceptor stimulation are not affected by anesthesia, it would seem that, in general, effects on intracellular calcium mobilization are much less than on influx of extracellular calcium at the doses of anesthetics that we used. The two anesthetics had comparable effects on constrictor responses in all 3 beds with the exception of the hindquarters which predominantly reflects skeletal muscle blood flow. Although chloralose/urethane did not affect vasoconstriction in this region, pentobarbital completely abolished responses to angiotensin II and noradrenaline. In a recent study, we have observed hindquarter vasodilatation as the only vasodilatory response to calcium-

entry blockers in conscious normotensive and hypertensive rats (Nievelstein et al, 1985). This suggests that, with regard to responses to calcium influx, the muscular vascular bed behaves differently from the others. The reason for the difference between PB and C-U observed in the present study is not clear but may depend upon relative importance of the effects of pentobarbital and chloralose/urethane on the two calcium mechanisms. Possibly, pentobarbital has more pronounced effects on calcium-influx dependent vasoconstriction than chloralose-urethane.

In conclusion, the present study indicates that anesthetic doses of both pentobarbital and chloralose/urethane differentially inhibit vasoconstrictor responses with regard to the nature of the constrictor agent. Furthermore, especially responses in the skeletal muscle vascular bed may depend upon the anesthetic used. This warrants further care in the interpretation of results from cardiovascular pharmacological experiments in which anesthetics are used.

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7. IN-VIVO EFFECTS OF SYNTHETIC ATRIOPEPTIN II (APII) ON MESENTERIC MICROVESSELS OF SHR

7.1 Introduction

Atrial natriuretic factors (ANF) are a group of small peptides that derive from the heart and which have pronounced natriuretic properties (DeBold et al, 1981; Maack et al, 1984; Needleman et al, 1985; Ballerman et al, 1986). Besides their renal effects, natural as well as synthetic ANF have also been shown to relax pre-contracted vascular smooth muscle from a variety of arteries in vitro (Atlas et al, 1984; Garcia et al, 1984; Misono et al, 1984; Winquist et al, 1984). In a previous study in spontaneously hypertensive rats (SHR), it was noted that infusions of the synthetic ANF atriopeptin II (APII) did not cause arterial vasodilation, but rather increased regional vascular resistances (Lappe et al, 1985). At the same time, cardiac output fell as a result of reduced cardiac filling pressures. Since this reduction of filling pressures persisted after bilateral nephrectomy, a (veno)dilator action on capacitance vessels for APII in vivo was proposed (Smits et al, 1985).

Therefore, in the present study, we investigated vascular effects of APII in SHR by comparing effects of systemically and intra-arterially applied APII both on the intestinal microvessels of the distal ileum and on mesenteric microvessels in vivo.

7.2 Experimental protocol

Rats (SHR), weighing between 330-370 g, were prepared for intravital microscopy of the mesentery or intestinal microcirculation as described in section 2.7. Fig. 7.1 depicts the general lay-out of the experiments. Catheters were implanted for measurement of mean arterial pressure (MAP) and heart rate (HR) and furthermore for systemic infusion of pentobarbital or atriopeptin II (APII). Surgical procedures were performed as described in detail in chapter 2. A small catheter was implanted in a small side branch of the superior mesenteric artery

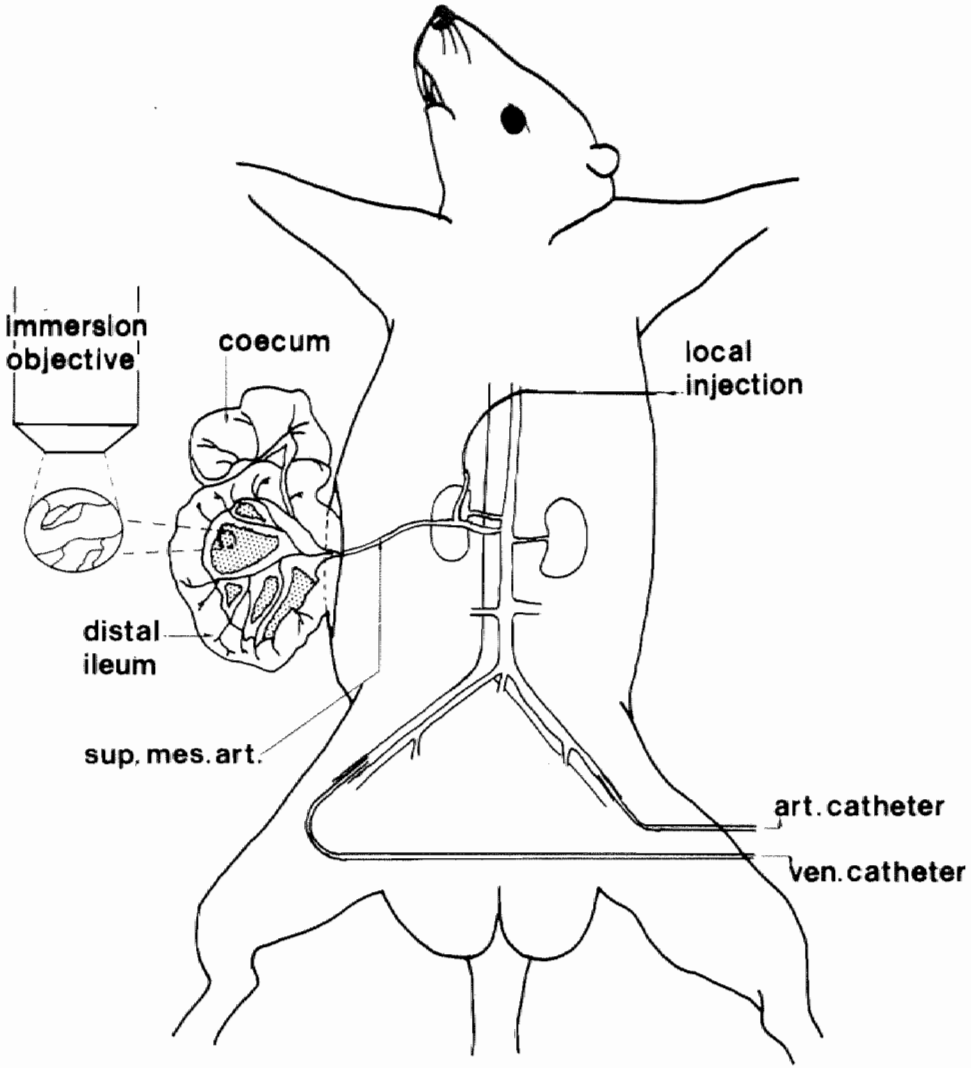

Fig. 7.1: General lay-out of the experimental set-up for the mesentery preparation.

in a subset of experiments (section 2.4). The preparation was inspected at low magnification with a Leitz long working distance objective (Leitz 4x). Side-branches which directly branched off from the mean feeding and draining vessels of the ileum were classified as second order vessels (A2 and V2, respectively). Second order vessels were video-documented with saltwater immersion objectives (SW 25 or SW 50).

Since the main vessels are surrounded by fat tissue which avoids proper (video) analysis of its side-branches, measurements were performed in the "empty" loops as close as possible to their branching points.

Vessels of the intestinal microcirculation were video-documented with a Leitz 4x objective. Diameter and red blood cell velocity measurements (V_{rbc}) were performed as explained in chapter 2.10.1 and 2.10.2.

Blood pressure and heart rate were measured via the left femoral artery (see section 2.3) and systemic drug infusions were applied via a femoral vein (see section 2.4). Atriopeptin II (APII) was infused i.v. while monitoring second-order microvessels in 8 animals. A control group of 6 rats was infused with saline.

Effects of intra-arterial (i.a.) infusions on second-order vessels were studied in 7 animals infused with APII and in 6 animals infused with saline. In all these experiments, only one A2 and one V2 vessel were studied.

A1 and V1 vessel responses of the intestinal microcirculation were studied during i.a. infusions of APII in 5 animals (14 A1 and 14 V1 vessels), whereas effects of saline were studied in 4 animals (9 A1 and 9 V1 vessels).

For infusions a ramp-type protocol was used, similar to the one we used before (Lappe et al, 1985; Smits et al, 1985). Thus, after an equilibration period of 30-45 min, base-line measurements for all parameters were made. Infusions were started at the lowest infusion rate (0.06 µg APII/kg.min for i.a. infusions or 0.25 µg APII/kg.min for i.v. infusions). Saline was infused in control animals at an identical volume flow rate (0.08 µl/min respectively 0.2 µl/min). MAP and HR were recorded throughout the course of the experiment. After 15

min, diameter and velocity measurements were made and the infusion rate was doubled. This procedure was repeated after 15 min.

All data are expressed as mean ± SE. Groups were compared using a oneway analysis of variance for unpaired observations (see chapter 2.11). Effects were considered statistically significant if $p<0.05$.

7.3 Results

Base-line values for mean arterial pressure (MAP), heart rate (HR), vascular diameters and red blood cell velocities (V_{rbc}) are summarized in table 7.1. MAP and HR did not differ significantly. Also, comparing the same order vessels, diameters and V_{rbc}s were similar in the groups.

7.3.1 Effects of systemically infused APII

Effects of saline and APII during i.v. infusions are shown in fig. 7.2. Saline did not substantially affect MAP and HR. Vascular diameters were also not affected. Effects on arteriolar V_{rbc} were variable, resulting in a 16±16% increase during infusion of 0.8 µl/min.

APII caused a dose-dependent fall in MAP (-41±5 mm Hg during infusion of 1 µg/kg.min). HR did not change statistically significantly. V_{rbc} decreased both in A2 (-33±10%) and V2 vessels (-34 ±8%). These decreases were significant as compared to the effects of saline ($p<0.05$). V2 diameter was not significantly affected by APII. A2 diameter decreased significantly (-12±5% during 1 µg/kg. min; $p<0.05$).

7.3.2 Effects of intra-arterially infused APII

Effects of i.a. infused saline and APII on MAP, HR and second-order vessels are summarized in fig. 7.3. Again, saline did not have any systematic effects. APII reduced MAP only at the highest dose (-32±9 mm Hg; $p<0.05$), which corresponds to the lowest dose infused i.v. The effects of this dose were not significantly different for the two routes. When given i.a., APII did not affect vascular diameters. However, as before both arteriolar and venular V_{rbc} decreased (-35±11%

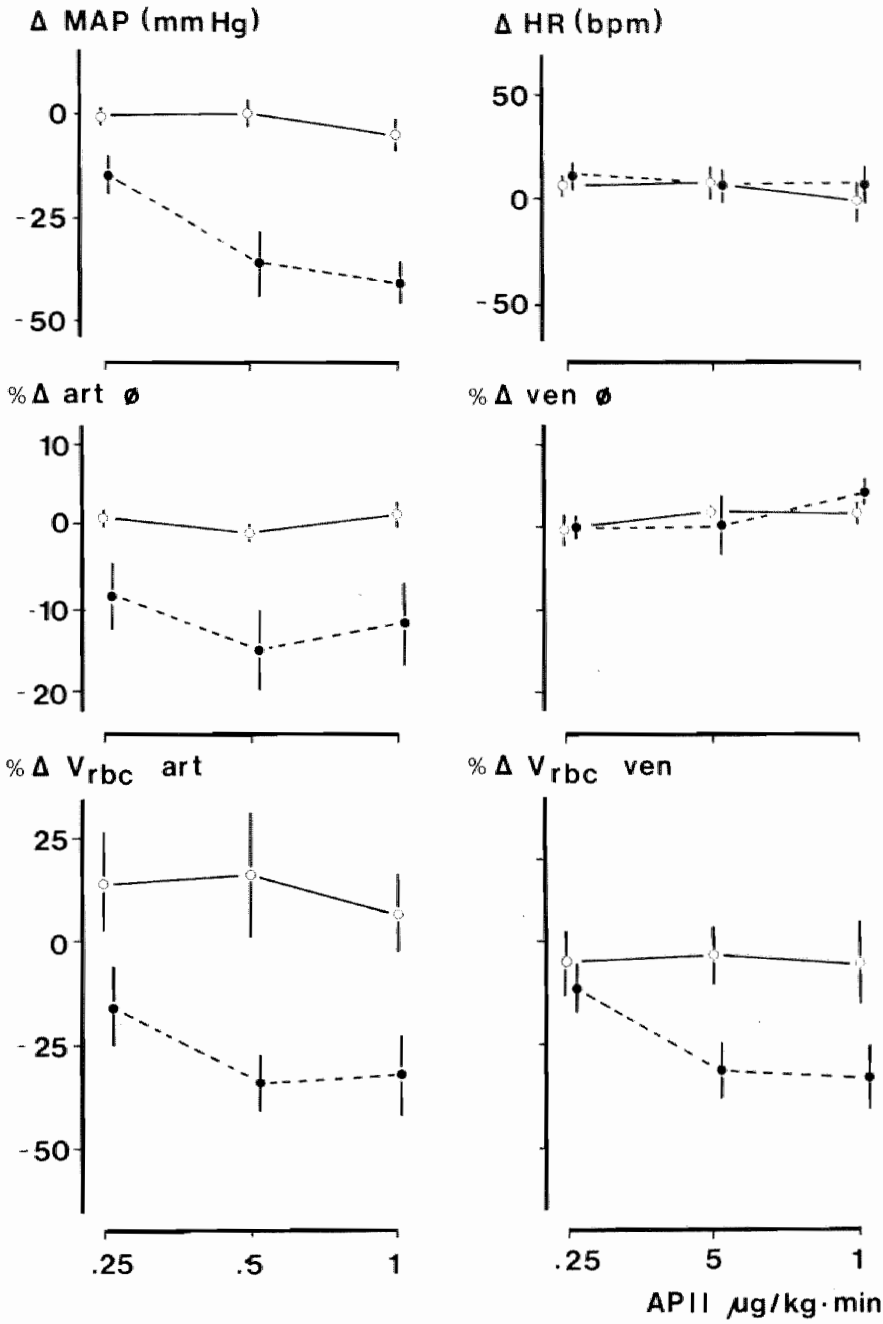

Fig. 7.2: Effects of saline (open symbols) and APII (closed symbols) on MAP, HR, A2 diameter, V2 diameter, and red blood cell velocities in A2 and V2 vessels during intravenous infusions. Significance in the difference between saline and APII infusions: *$p<0.05$.

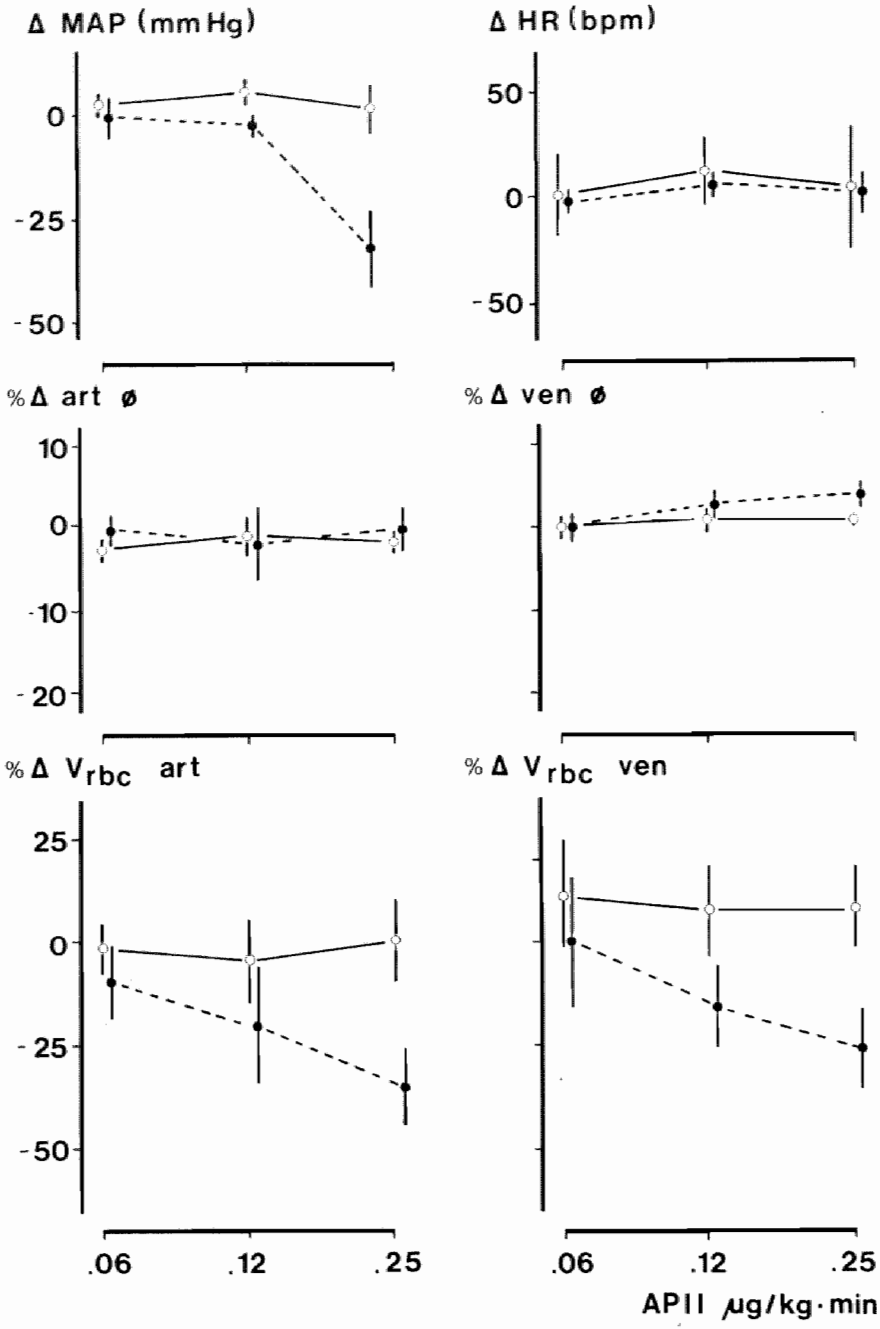

Fig. 7.3: Effects of saline (open symbols) and APII (closed symbols) on MAP, HR, A2 diameter, V2 diameter, and red blood cell velocities in A2 and V2 vessels during intra-arterial infusions. Significance in the difference between saline and APII infusions: *$p<0.05$.

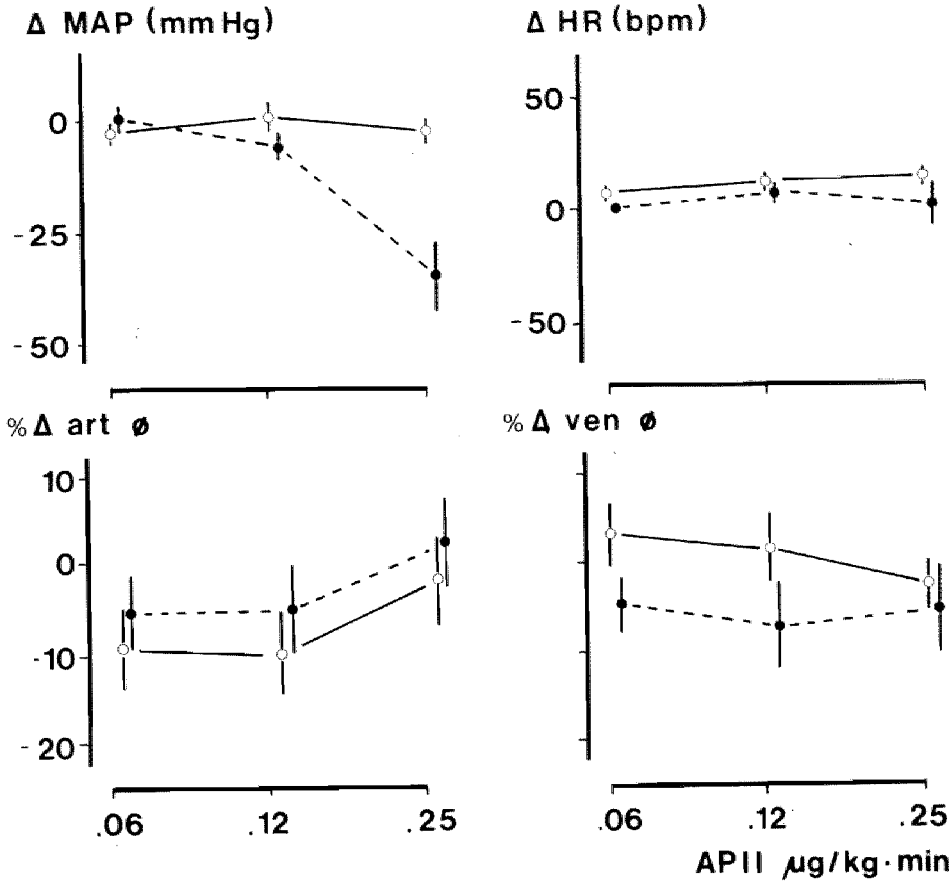

Fig. 7.4: Effects of saline (open symbols) and APII (closed symbols) on MAP, HR, Al diameter, and Vl diameter during intra-arterial infusions. Significance in the difference between saline and APII infusions: *$p<0.05$.

and -26±10%, respectively, during 0.25 μg/kg.min; $p<0.05$ as compared to saline). Effects on V_{rbc} were again not significantly different at this dose, with regard to the route of administration.

Effects of i.a. infused APII and saline on MAP, HR and diameters of first-order arterioles and venules in the ileum of a separate group of animals are summarized in fig. 7.4. Also in this group, APII decreased MAP only at the highest infused dose (-34±8 mm Hg). Neither A1 and V1 diameters were significantly affected by i.a. infusions of APII.

Table 7.1: Start values for the groups (mean ± SEM).

Group	MAP (mm Hg)	HR (bpm)	art.diam. (μm) A2 vessels	ven.diam. (μm) V2 vessels	V_{rbc},art. (mm/s)	V_{rbc},ven. (mm/s)
i.v. saline	177±2	325±13	26.7±3.5	56.6±7.2	8.7±1.9	2.0±0.4
i.v. APII	165±6	316±8	21.8±3.4	41.6±8.0	8.5±2.7	2.4±0.8
i.a. saline	161±6	305±15	19.7±2.9	38.3±5.9	7.6±1.6	2.2±0.4
i.a. APII	152±7	290±11	18.7±1.5	38.4±2.9	8.2±1.7	2.2±0.2

Group	MAP (mm Hg)	HR (bpm)	art.diam. (μm) A1 vessels	ven.diam. (μm) V1 vessels	V_{rbc},art. (mm/s)	V_{rbc},ven. (mm/s)
i.a. saline	168±7	310±7	69±2	138±7	-	-
i.a. APII	160±5	290±11	68±4	132±9	-	-

7.4 Discussion

Although several investigators have noted a vasodilator action of ANF in isolated, precontracted blood vessels (Atlas et al, 1984; Garcia et al, 1984; Misono et al, 1984; Winquist et al, 1984), we did not observe vasodilation in intact, conscious SHR during infusions of the synthetic ANF APII, but rather vasoconstriction (Lappe et al, 1985). Reductions of cardiac filling pressures did, however, suggest a venodilator effect (Lappe et al, 1985; Smits et al, 1985). The present study was designed to further characterize the effects of APII on the vasculature in vivo. We therefore studied the effects of both systemically and locally administered APII on the mesenteric microcirculation of SHR.

During i.v. infusions of APII, blood pressure fell. This effect was associated with a reduction of A2 diameter which parallelled the fall in MAP. V2 diameters did not change. Also V_{rbc} fell in both arterioles and venules, which is in accordance with the previously found reduced splanchnic blood flow (Lappe et al, 1985).

In order to discriminate between direct and indirect effects of APII, low-dose infusions were given directly into the supplying artery of the vessel beds under study in a second group of SHR. Here, we noted no effects on arteriolar (A1 and A2) and venular (V1 and V2) diameters. V_{rbc} was reduced only at the highest infusion rate, at which also blood pressure was reduced. These results suggest that the reduction of arteriolar diameter during infusion of APII in the first part of the study does not result from a direct effect on the mesenteric vasculature. This is in accordance with a previous report, showing a lack of effect of ANF on isolated mesenteric microvessels in vitro (Aalkjaer et al, 1985). Arterioles in that study were 5-10 times larger in diameter (200 μm) than the ones we studied here.

Most likely arteriolar constriction during i.v. infusion of APII results from reflex effects. In a previous study, Lappe and co-workers (1985) showed that in conscious SHR chemical sympathectomy prevents the vasoconstrictor responses to APII in the mesenteric circulation. Possibly, the sympathetic nervous system also mediated vasoconstriction in our study, although primary cardiovascular reflexes like the

sinoaortic baroreflex should be expected to be damped in our anesthetized preparation (Cox and Bagshaw, 1979). We did not further pursue this.

At the highest local infusion rate, corresponding to the lowest given intravenously, we did not observe vasoconstriction, although MAP and V_{rbc} were reduced significantly. In fact, blood pressure was reduced by 35 mm Hg. During i.v. infusions, similar reductions in MAP were associated with 10-15% reductions of A2 diameter. This suggests that the high APII levels achieved locally in the mesenteric circulation were capable of antagonizing the expected reflex vasoconstriction in a way comparable to the effect of ANF noted on precontracted vessels (Atlas et al, 1984; Garcia et al, 1984; Misono et al, 1984; Winquist et al, 1984).

We have previously shown that the blood pressure reduction during infusion of APII in SHR depends upon a fall in cardiac output which is, in turn, associated with reduced central venous and left atrial pressures (Lappe et al, 1985). Since this latter effect persisted after bilateral nephrectomy, we suggested a venodilator action of APII in SHR (Smits et al, 1985). In the present study, we did not observe an actual dilation of venules. This may suggest that dilation occurs only in larger capacitance vessels, or in beds other than the splanchnic circulation. Alternatively, the reduction of cardiac filling pressures may depend upon a reduction of blood volume through a route that does not depend upon diuresis. In this regard, it has recently been suggested that ANF may cause a shift of water from the intravascular space to the extravascular space through increased capillary filtration (Trippodo et al, 1986). Data in the present study do, however, not corroborate this suggestion. The 10-15% reduction of arteriolar diameter results in a 40-60% increase of (precapillary) arteriolar resistance, assuming a cubic dependence of resistance on microvascular diameter (Mayrovitz and Roy, 1983). The lack of venular diameter change would suggest a decrease in capillary pressure during i.v. infusion of APII, especially since flow fell at the same time.

Thus, although the present data do not allow a definite conclusion on the effect of ANF on capillary filtration, they do suggest that the hydraulic situation is in favor of a decrease, rather than an

increase of capillary filtration. Possibly, however, ANF may increase vascular permeability. In the next chapter the effect of ANF on microvascular permeability will be investigated.

In conclusion, the present study indicates that APII has no direct effect on ileum and mesenteric microvascular diameters in SHR in vivo. We did not obtain direct evidence for venous dilatation. The results suggest that the hydraulic conditions in the vascular bed between A2 and V2 vessels are not in favor of increased capillary filtration.

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8. EVALUATION OF MACROMOLECULAR LEAKAGE AS A POSSIBLE MECHANISM FOR THE BLOOD PRESSURE LOWERING EFFECT OF ATRIAL NATRIURETIC PEPTIDE (102-126 hANF)

8.1 Introduction

The physiological role and mechanism of atrial natriuretic peptides (ANF) in cardiovascular homeostasis are poorly understood. Most studies agree on the blood pressure lowering effect being partly explained by its potent diuretic and natriuretic activity.

The (extrarenal) vascular effects are controversial. A decrease in total peripheral resistance occurs as a consequence of arteriolar dilation. The hypotensive effect of ANF, which has been observed in a variety of hypertensive models, has been assumed to be related to a vasorelaxant effect in vitro (Atlas et al, 1984; Garcia et al, 1984; Needleman et al, 1985; Thompson and Webb, 1986). However, based on in-vivo studies (Lappe et al, 1985; Volpe et al, 1986; Pegram et al, 1986) as well as on the data presented in the previous chapter obtained from microvascular studies, it seems unlikely that arteriolar dilation occurs. The arterioles are not the primary site of action of ANF. Rather the hypotensive effect is associated with an enhanced arteriolar constriction and an increase in total peripheral resistance (Lappe et al, 1985, 1986; Criscione et al, 1987). Blood pressure is reduced through reduced cardiac filling pressures and, consequently reduced cardiac output. The fall in venous pressure during continuous infusions of ANF could not be explained by a venous dilation (chapter 7). Both mesenteric as well as intestinal venules showed constant diameters, while cardiac filling pressures and thus cardiac output decreased.

Alternatively, decreased filling pressures may result from a decreased circulating blood volume as a consequence of an enhanced sodium and fluid excretion by the kidneys or by a fluid shift toward the interstitium. Even in bilaterally nephrectomized rats ANF decreases cardiac filling pressures and blood pressure (Smits et al, 1985) which indicates that the renal action is not a prerequisite for the blood

pressure decrease.

From human and animal experiments, it has been suggested that infusions of very low doses of ANF increase the hematocrit (DeBold et al, 1981; Biollaz et al, 1986; Flückiger et al, 1986), although others could not confirm this (Lappe et al, 1985). An increase in hematocrit in humans was noticed before the onset of urinary losses of fluid and electrolytes (Biollaz et al, 1986). The hypotension was attributed to an increased capillary permeability with fluid shifts. This was also suggested by others in the renovascular hypertensive model (Flückiger et al, 1986). Possible other sites of action of ANF besides renal have to be included. Experimental evidence suggests that ANF may regulate water and ion transport in tissues such as the intestines, ciliary bodies and choroid plexus (review, see Cantin and Genest, 1985).

The present study focusses on the role of ANF on local fluid transport and homeostasis within the rat mesentery microcirculation. ANF was continuously infused to assess whether the blood pressure decrease was associated with a simultaneous extravascular appearance of fluorescent macromolecules as an indicator of increased microvascular permeability. In addition, microvascular diameters and red blood cell velocity were measured to determine vascular responses.

8.2 Experimental protocol

8.2.1 Animals

Wistar rats, weighing between 300-375 g, were anesthetized with sodium pentobarbital and catheters inserted to measure mean arterial blood pressure (MAP) and heart rate (HR) (see section 2.2 and 2.3, respectively).

The left femoral vein was used for a continuous infusion of sodium pentobarbital (see section 2.2). Depending on the experimental protocol, ANF was either applied systemically via the right femoral vein (section 2.4.1) or locally via a small side-branch of the superior mesenteric artery (see section 2.4.2).

8.2.2 Intravital microscopy

Rats were prepared for intavital microscopic observation of the mesentery microcirculation (section 2.7.1). Vessels were classified according to their hierarchical branching patterns. The centripetal mode of classification allowed for a functional description of the microvasculature. The arteriole which branches off perpendicular to the feeding vessel of the intestine was classified as second order (A2). Third and fourth order side-branches were classified as A3 and A4 vessel respectively. The same nomenclature was used on the venous side of the circulation. Venules were classified as V2, V3 and V4, respectively. Arterioles and venules were studied using transillumination (see section 2.9.1 and 2.10.1) and observed with a SW50 objective.

Velocity measurements were only performed in A2 and V2 vessels (see section 2.10.2). It was found that in a typical preparation these vessels suppply and drain one vascular bed and velocity measurements thus reflect total microvascular blood flow.

In a separate series of experiments, microvascular permeability was studied using fluorescence microscopy (see section 2.9.1).

FITC-BSA (Fluorescein isothiocyanate, bovine serum albumin, Sigma, St. Louis, MO, USA) was infused intravenously (i.v.) within 3-5 min at a dose of 200 mg/kg. FITC-BSA was dissolved in 0.5 ml physiological saline (0.9% NaCl). The luminescent vessels were observed with a Leitz 4x objective and video-documented as explained in section 2.9.1. Within one field of view on the video monitor, large areas of the vascular bed could be monitored (arterioles, capillaries and venules). Only areas were selected with no sign of spontaneous leakage. In case lymph vessels were present within the mesentery microcirculation these were video-documented as well. The microvascular permeability could be assessed by counting the number of leaky spots following drug infusion. The experimental protocol was always ended by superfusion with histamine (10 µg/ml) to determine the ability of the preparation for protein leakage (De Clerck et al, 1985). Microvascular leakage of macromolecules was studied following systemically administered 102-126 hANF while in the control group saline was infused.

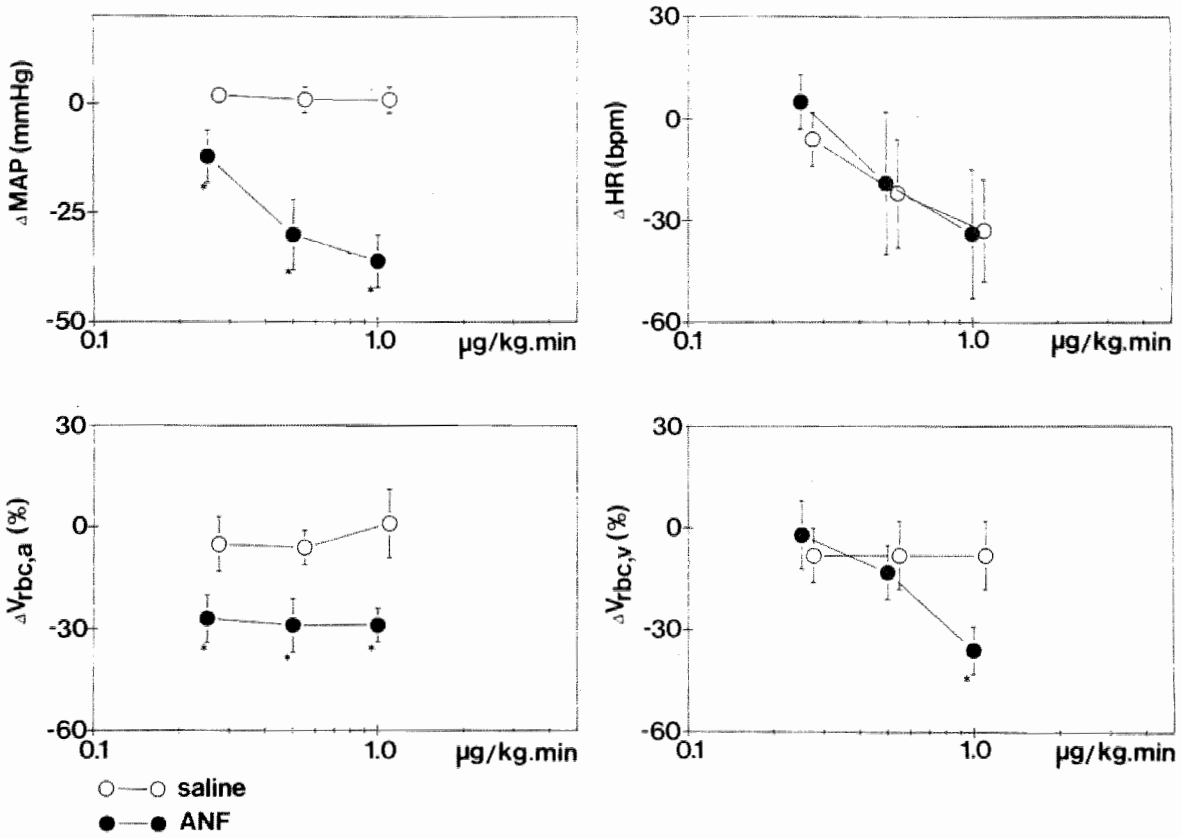

Fig. 8.1: Effects of saline (open symbols) and (102-106) hANF (closed symbols) on mean arterial pressure (MAP), heart rate (HR). Arteriolar (Vrbc,a) and venular (Vrbc,v) red blood cell velocities during continuous intravenous infusions. Significance in the difference between saline and (102-106) hANF infusion: *$p<0.05$.

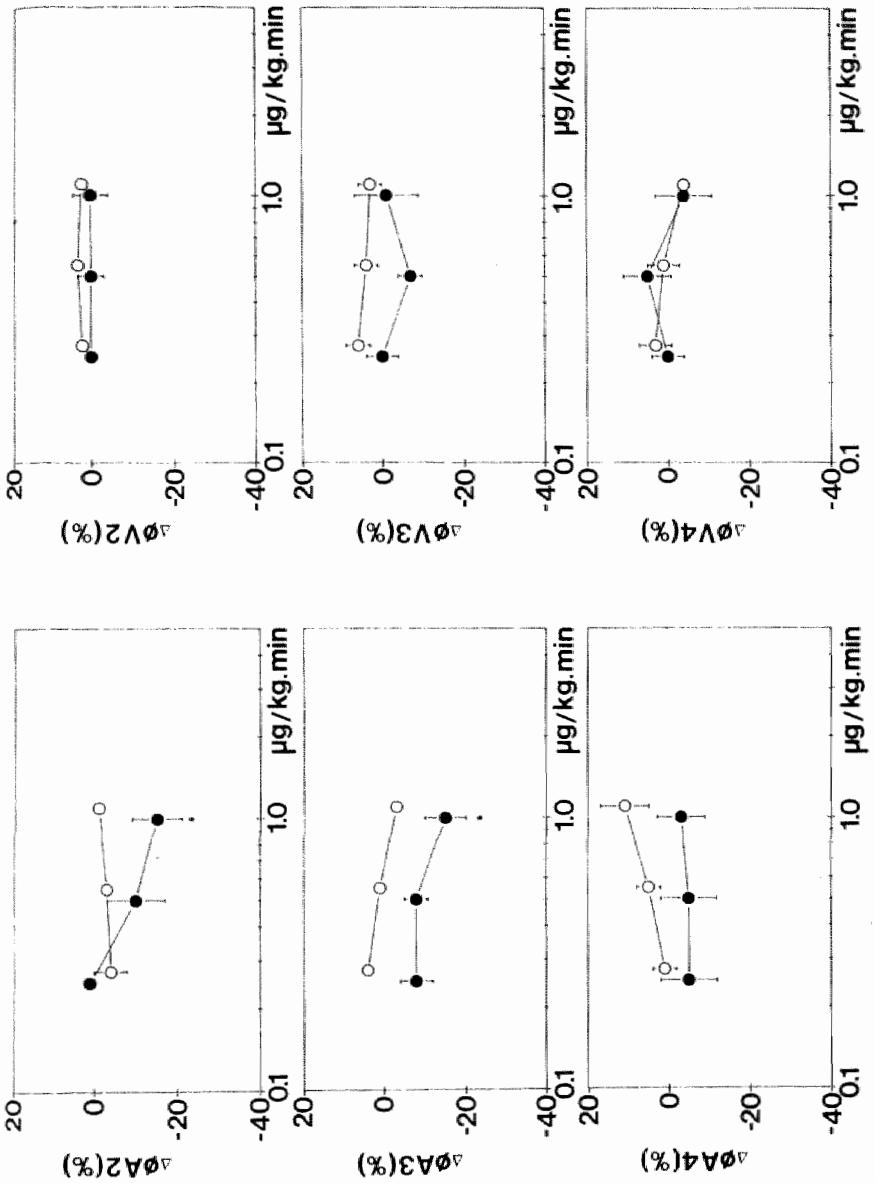

Fig. 8.2: Effects of saline (open symbols) and (102-106) hANF (closed symbols) on arteriolar (A_2, A_3, A_4) and venular (V_2, V_3, V_4) diameters during continuous intravenous infusions. Significance in the difference between saline and (102-106) hANF infusion: *$p<0.05$.

8.2.3 Drug dosage

ANF (102-106 hANF; WY 47.663) was infused i.v. in 7 rats in a dose of 0.25-0.5-1.0 µg/kg/min. In 8 rats only the vehicle solution (saline) was infused i.v. as control. Local intra-arterial infusion of ANF was performed in 7 rats and an equal number of rats received saline as control. For local drug delivery, a lower dosage was used (0.0625-0.125-0.25 µg/kg/min).

For infusions, a ramp-type protocol was used (Lappe et al, 1985). Baseline micro- (diameter, V_{rbc}) and macro hemodynamic parameters (MAP, HR) were determined during a 5-min period.

Increasing doses of ANF or vehicle solution (saline) were infused. The doses were increased at 15-min intervals. Changes in the hemodynamic parameters were recorded at each dose level after a steady-state condition was achieved (15-20 min). Measurements were completed within 5 min.

Protein extravasation during infusion of ANF was studied in 8 rats and only the lowest and highest i.v. dosages were used. In 6 rats saline was infused systemically. These animals served as a reference group.

All data are expressed as mean $\pm$ SEM. Statistical analysis of the data was carried out, using one-way analysis of variance with repeated measures.

8.3 Results

Baseline values for mean arterial pressure (MAP), heart rate (HR), and vascular diameters and red blood cell velocity (V_{rbc}) are summarized for each subset of experiments in table 8.1.

8.3.1 Effects of systemically infused ANF

Effects of saline and ANF during i.v. infusion are presented in figure 8.1 and 8.2. Saline infusion did not affect vascular diameters or V_{rbc}. During the course of the experiment, MAP remained constant while HR decreased slightly. ANF caused a dose-dependent fall in MAP. V_{rbc} decreased both in A2 and V2 vessels.

Table 8.1: Base-line values for mean arterial pressure (MAP), heart rate (HR), and vascular diameters and red blood cell velocity (V_{rbc}).

	i.v. saline		i.v. ANF		i.a. saline		i.a. ANF	
	N	start value	N	start value	N	start value	N	start value
MP (mm Hg)	8	119±5	7	123±7	7	112±7	7	129±9
HR (bpm)	8	305±13	7	298±14	7	303±12	7	323±20
$V_{rbc,a}$ (mm/s)	7	3.8±0.5	6	4.6±1.0	7	8.3±2.1	7	6.6±2.5
$V_{rbc,v}$ (mm/s)	7	1.7±0.3	6	3.3±0.9	7	3.0±0.7	7	2.2±0.8
Ø A2 (µm)	8	25.2±2.9	6	24.4±3.4	7	27.0±3.3	7	30.4±4.5
Ø A3 (µm)	9	13.8±1.3	6	12.2±3.7	10	14.0±0.9	8	13.0±1.6
Ø A4 (µm)	9	9.4±0.8	5	8.2±0.6	7	9.6±0.7	5	9.3±1.6
Ø V2 (µm)	9	46.0±8.1	6	46.1±5.6	7	45.3±6.6	6	46.6±7.5
Ø V3 (µm)	9	18.7±3.5	5	20.5±4.1	9	19.9±3.3	8	24.0±3.9
Ø V4 (µm)	8	10.8±1.8	5	11.8±1.9	7	12.3±1.9	6	16.0±3.2

Systemic infusion of ANF did not affect HR. During i.v. infusion of ANF arteriolar diameters decreased. The most profound decrease was seen in the larger arterioles (A2 and A3). Differences were statistically significant for both arteriolar-order categories only for the highest dosages. Venular diameters remained constant during i.v. ANF infusion. This was observed for all vessel order levels.

8.3.2 Effects of intra-arterially infused ANF

Effects of i.a. infused ANF and vehicle solution on MAP, HR and arterioles and venules are presented in figure 8.3.and 8.4. Although ANF was infused at lower dosages, MAP decreased in a dose-dependent manner. HR was not affected. Local infusion of ANF resulted in a maximal decrease of approximately 50% of arteriolar and venular V_{rbc} at

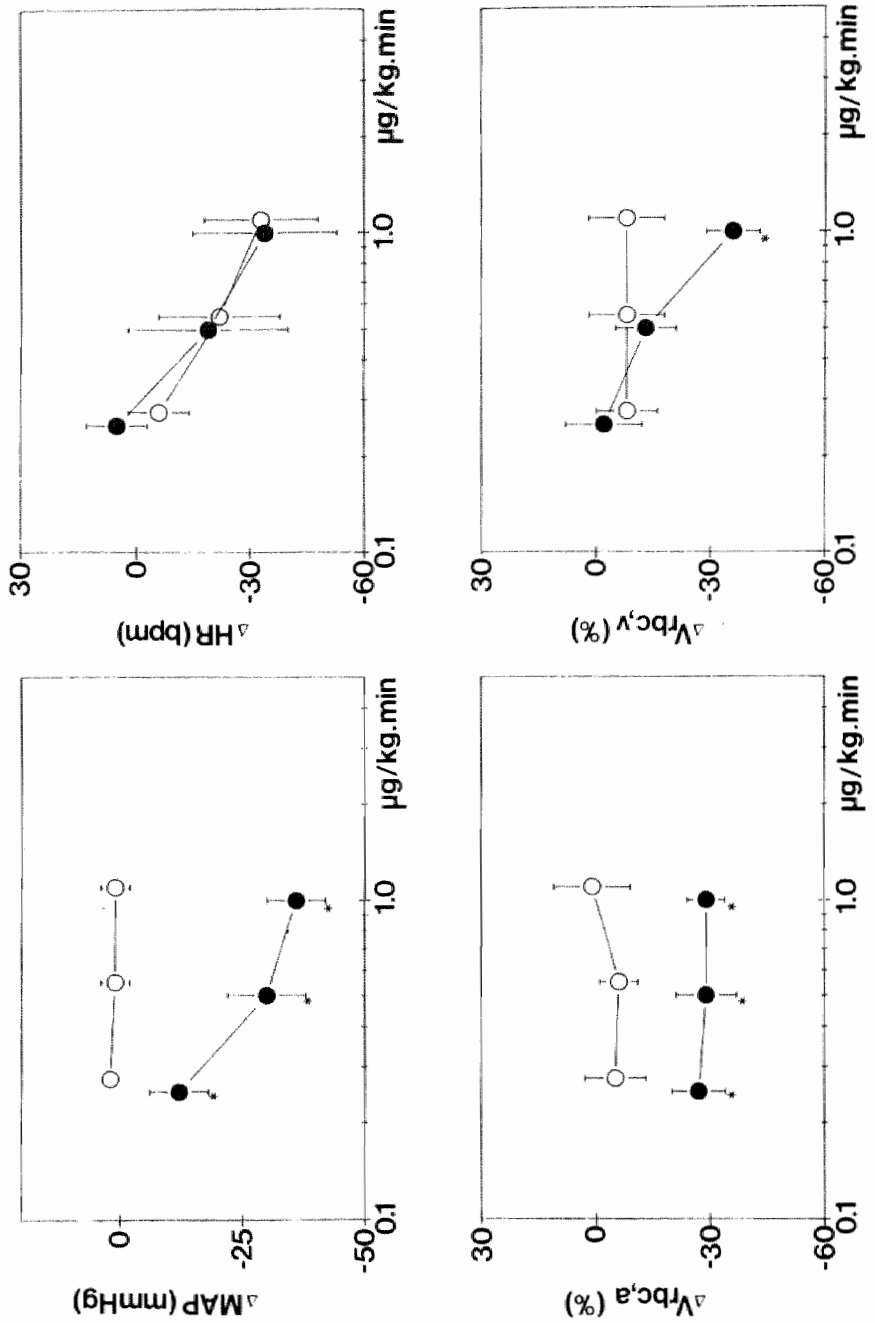

Fig. 8.3: Effects of saline (open symbols) and (102-106) hANF (closed symbols) on mean arterial pressure (MAP), heart rate (HR), arterial (Vrbc,a) and venular (Vrbc,v) red blood cell velocities during continuous intra-arteriolar infusions. Significance in the difference between saline and (102-106) hANF infusion: *$p<0.05$.

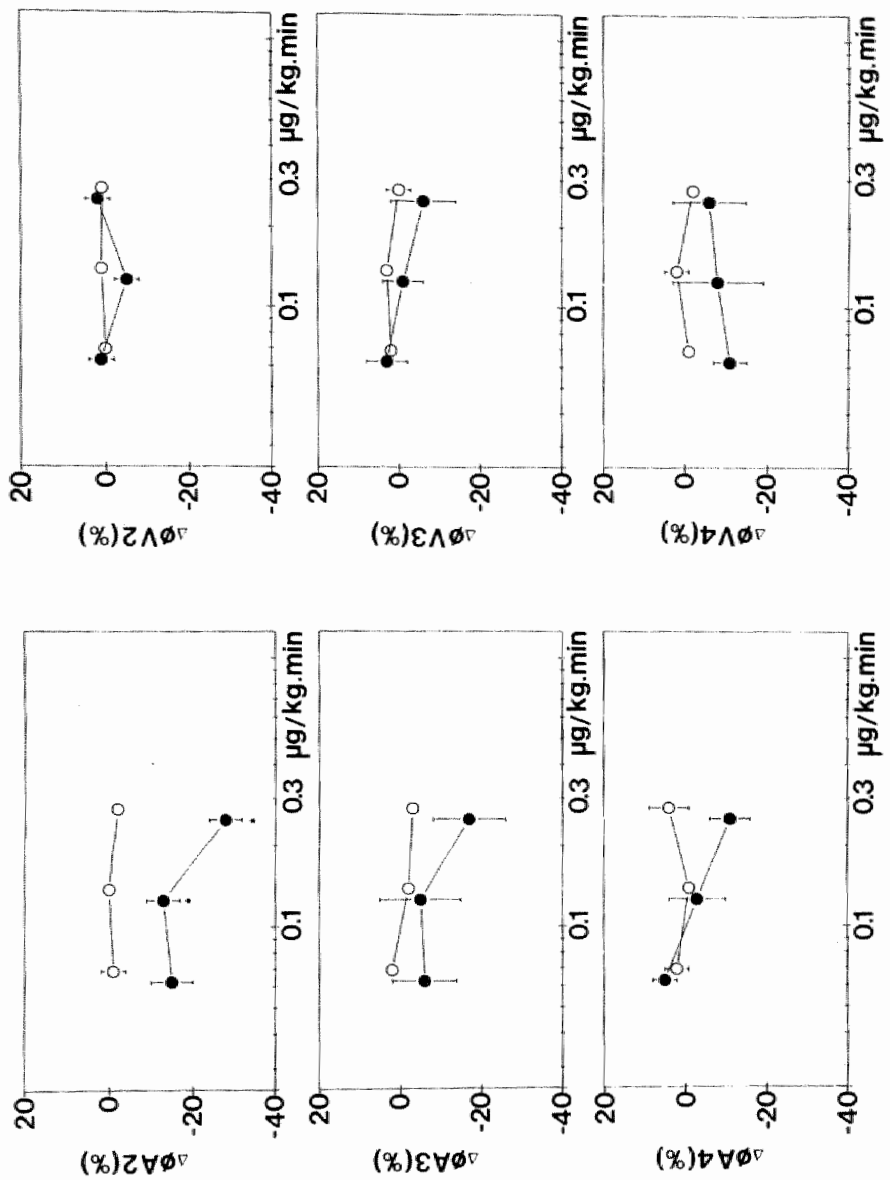

Fig. 8.4: Effects of saline (open symbols and (102-106) hANF (closed symbols) on arteriolar (A_2, A_3, A_4) and venular (V_2, V_3, V_4) diameters. Significance in the difference between saline and (102-106) hANF infusion: *$p<0.05$.

the highest infusion rate. Arteriolar constriction at several order levels was observed but this reached statistical significance only for the A2 vessels following 0.125 and 0.25 µg/kg/min ANF i.a. Venular diameter changes were not seen.

8.3.3 Effects of i.v. administered ANF on macromolecular leakage

In none of the experiments, despite a considerable decrease in MAP (table 8.2), did ANF affect macromolecular leakage, evidenced by the absence of extravascular fluorescent spots. Notably, plasma leakage did occur when the mesentery preparation was exposed to topically applied histamine. Following topical histamine, the leakage first developed rapidly at the postcapillary venules initially seen as multiple circular areas with intense fluorescence. Approximately 5 min after administration, the leakage became massive and the avascular areas of the mesentery were fluorescent as well. Histamine did not have any measurable effect on MAP. Lymph vessels were non-fluorescent both in the control situation and following treatment with ANF. Following topical histamine lymph vessels became fluorescent.

Table 8.2: Effects of saline and ANF on MAP and HR in rats used for the study on capillary protein extravasation.

	N	Start value	0.5	2.0 µl/min saline
MAP	6	125± 5	-1± 1	-2± 1
HR	6	322± 8	10± 1	11± 1
			0.25	1 µg/kg.min ANF
MAP	8	118± 3	-10± 1	-26± 4
HR	8	288±11	-2± 6	-9± 9

8.4 Discussion

The major objective of this study was to investigate the effect of ANF on capillary filtration processes by means of fluorescent macromolecules. The major finding of this study is that systemic infusions of ANF did not cause an enhanced leakage of macromolecules. This finding is important in relation to the effects of ANF on the regulation of blood pressure. Our data suggest that local fluid shifts are not involved in the hypotensive response following atrial natriuretic peptides. The enhanced arteriolar constriction and absence of venular diameter changes following ANF administration are in accordance with earlier observations presented in the previous chapter.

The mesentery has a unique hydraulic condition to study capillary filtration because microvascular pressures are higher than in other vascular beds (Bohlen and Gore, 1984). The intestine forms an in-series coupled network system that drains directly into the liver. It is likely that any change with regard to the altered microvascular permeability should first be reflected by macromolecular leakage in the mesentery.

During systemic infusions of ANF, blood pressure fell. This effect was associated with a diameter reduction of second and third order arterioles. No change of venular diameters was observed while V_{rbc} decreased in both arterioles and venules. These observations are in accordance with previous studies (Lappe et al, 1985; Criscione et al, 1987). In these studies in rats, splanchnic blood flow decreased and vascular resistance increased following administration of atrial natriuretic peptides (APII).

The decrease in cardiac output observed in other studies (Lappe et al, 1985) was explained by lowered cardiac filling pressures and venous return possibly by venous dilation. Under resting conditions (skeletal muscle), venules have a considerable vascular tone (Damon and Duling, 1986). Thus, assuming that also mesenteric venules have a resting tone our data do not corroborate this hypothesis although it still remains possible that larger venules or veins more downstream are involved and exhibit dilation.

The hemodynamic effects of atrial peptides depend on the mode of

their administration. Lappe et al (1986) recently showed that in conscious rats atrial peptides given as a bolus injection decreased renal and splanchnic vascular resistance. If a comparable blood pressure reduction was achieved by sustained infusions, regional vascular resistances increased. It is as yet unknown what the primary underlying mechanism is. This vasoconstriction appeared to be mediated through an increased sympathetic tone rather than through a direct vasoconstrictor action of the peptide because sympathetic denervation abolished the response (Lappe et al, 1985). This fits well in our observations demonstrating an arteriolar constriction during continuous infusions of ANF.

To avoid the possible involvement of systemic (reflex) effects, local drug administration was performed. During local infusion, no evidence was obtained for any vasorelaxant effect. The most prominent feature was an actual decrease in arteriolar diameter of second order arterioles. ANF failed to cause a venodilation in this preparation also.

An unexpected finding was that low-dose local infusion of ANF decreased blood pressure. This may be related to a possibly excitatory action of atrial peptides on chemosensitive receptors within the splanchnic bed. These receptors may elicit inhibiting afferent signals towards the cardiopulmonary region or the central nervous system (Longhurst, 1984). Secondly, it is conceivable that atrial peptides could interfere with the secretion and action of vaso-active peptides of the gastro-intestinal tract (Burnstock and Griffith, 1983). Thirdly, the effect might be attributed to the unique pharmacological properties of ANF. In the previous chapter, it was found that local administration of low dose APII did not lower systemic blood pressure. The design of the present experiments does not allow any conclusions to the nature of the differential effects.

It was hypothesized that atrial peptides are involved in the regulation of fluid and electrolyte balance of peripheral tissues. This could be a potential mechanism for the hypotensive action of atrial natriuretic peptides, promoting an increased capillary filtration. In order to demonstrate an effect of ANF on microvascular permeability, macromolecular leakage was studied. Even the highest doses

failed to cause an enhanced leakage nor did an enhanced protein transport by lymph vessels occur, indicating that ANF does not promote fluid and protein shifts towards the extravascular space in this part of the circulation. Theoretically, an increased capillary protein leakage may be achieved by an increased number of perfused capillaries without any change of microvascular permeability of individual capillaries. However, capillary recruitment has not been demonstrated for the mesentery and does hardly occur in the intestine (Bohlen, 1983; Harper et al, 1985).

Recently it was demonstrated that the gastro-intestinal tract possesses specific high affinity binding sites for atrial natriuretic peptides (Mantyh et al, 1986). The intestine is involved in fluid and electrolyte reabsorption (Granger et al, 1984) and could be a primary site of action for atrial peptides thus influencing hydrostatic and oncotic pressures. An increased interstitial volume may result in an increased hydrostatic tissue pressure. This should preferentially drive fluid into the lymphatics because the hydraulic conductance of these vessels is greater than that of capillaries (Granger et al, 1984). Since the lymph vessels we studied originate close to the intestinal wall and were non-fluorescent, the hypothesis of a specific effect of ANF upon intestinal fluid and protein homeostasis has to be abandoned.

In conclusion, continuous infusion of ANF resulted in a dose-dependent blood pressure decrease. The hypotensive effect could not be explained by a direct vasodilatory effect on mesenteric microvessels nor by en enhanced vascular capillary filtration within the same tissue. It remains to be established whether the blood pressure decrease can be explained by a direct effect on larger vessels and/or other tissues.

8.5 References

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9. GENERAL DISCUSSION

In the previous chapters experimental data have been presented about microvascular changes during the development and maintenance of spontaneous hypertension as well as on the effects of neurogenic and humoral influences on microvascular dynamics under these circumstances.

The development of high blood pressure in spontaneously hypertensive rats (SHR) has been explained on the basis of a renal mechanism to retain fluid leading to volume-loading or a primary change in vascular resistance (for references see chapter 1). Since the developmental phase of spontaneous hypertension is characterized by an uneven fractional distribution of flow to the major organs, the question has been raised whether the increased vascular resistance is distributed uniformly over the vascular bed. Although whole-organ experiments have provided evidence that resistance is predominantly increased in the terminal part of the circulation the necessity for microvascular studies is obvious.

Changes in vascular resistance may be caused by structural or functional adjustments of the vascular bed (Bohlen, 1986). Folkow et al (1983) have hypothetized that changes in vascular morphology should prevent vessels from normal vasodilation and thereby increase the wall thickness to lumen ratio resulting in an increased blood pressure. Although wall thickness was not measured in our study the finding that the maximum diameter obtained was not different in WKY and SHR in all types of arterioles analyzed suggests that adaptive changes of the vessel wall in muscular tissue do not play a major initiating role in the aetiology of spontaneous hypertension. This has also been found by other investigators (Henrich et al, 1978; Bohlen, 1983; Miller et al, 1987).

The data as obtained in the mesentery and intestine by Henrich and Bohlen, indicate that arteriolar wall hypertrophy may occur, but that hypertrophy is not an obligatory mechanism in the development of hypertension. In another model of hypertension, the one-kidney one-clip renal hypertensive rat, the wall thickness to lumen ratio of

increased with time, thus slowing the
react adequately to blood pressure in-
).

still remains why increases in wall
may occur in terminal arterioles and multi-
occurring, are mainly restricted to larger
diameters of more than 100 μm (Baumbach,
; Lee, 1983; Brayden et al, 1983; Miller

circulatory events in the young SHR is the
on increased cardiac output and redis-
ributed muscle (Smith and Hutchins, 1979;
speculate that the increased flow would
the tissues which would in turn prevent
culature and lead to rarefaction. Rare-
gained as a basic abnormality in the SHR
scular resistance (Hutchins and Darnell,
rich and Hertel, 1982; Chen et al, 1981;
al, 1982).

long-term autoregulatory response to
comes from a study of Regan and Hirsch-
on of the main feeding artery of the rat
durable increase in the number of arte-
. The same percentage of arterioles was
a resting state like in the non-banded

by counting the number of vessels using
ques. They concluded that arteriolar
ures modulate arteriolar density. The
e for arteriolar rarefaction in the SHR

study further support this concept. In
llary arterioles perfused is diminished
ol and vasodilated state. This in favor
change in the vascular architecture.
nderlying the increased resistance and,
n blood pressure, may be a diminished

growth.

The contribution of oxygen to an elevated total peripheral resistance in SHR has been suggested by Walsh and Tobia (1982) who reported that oxygen has to be available for subclavian vascular resistance to be elevated in young (8 weeks old) cord-transsected SHR. In addition, repeated blood flow increases appear to produce an enhanced autoregulatory constrictor response in young and cord-transsected SHR in comparison with WKY. The enhanced response of SHR vessels to oxygen availability (Lombard et al, 1984) suggests that arterioles of the hypertensive animals will increase vascular resistance more than those of WKY when oxygen availability is increased. Furthermore, tissue oxygen uptake in the rat is flow-dependent (Banchero, 1987). Therefore, under conditions where tissue pO_2 is not maintained at a relatively low level, local long-term vascular control mechansims could contribute to the elevated vascular resistance in SHR.

The role of oxygen in the development of hypertension has been known for many years. Exposure to high altitude with low ambient oxygen tensions decreases the prevalence of hypertension in humans or experimental animals and reduces blood pressure to near normal levels (Marticornes et al, 1969; Banchero, 1975; Ruiz et al, 1977; Prewitt et al, 1986). Chronic hypoxia elicits microvascular changes associated with a reduced vascular resistance, including reduced levels of vasoconstriction and prevention of arteriolar rarefaction (Prewitt et al, 1986). The mechanism by which hypoxia reduces vascular resistance is not fully understood but a number of experiments points towards the role of adenosine, an endogenous vaso-active metabolite with angiogenetic activity (Banchero, 1975; Tornling et al, 1978, 1980; Mattfeld and Hall, 1983; Hudlicka et al, 1984), an altered sodium metabolism (Behm et al, 1984), an altered catecholamine metabolism (Barbarash et al, 1982) or may be related to a metabolic adaptation in the SHR (Henley and Tucker, 1987).

In the adult SHR with a normal distribution of cardiac output Evenwel et al, 1983), arterioles with a diameter of 80-150 μm, which are responsible for a considerable part of the increased resistance, did exhibit reduced diameters as compared to WKY. During the maintenance phase of spontaneous hypertension vascular resistance is in-

creased in the terminal vascular bed because of a lower number of precapillary arterioles and a smaller lumen size of larger arterioles, as compared to WKY. This may have important consequences for the development of future antihypertensive drugs if a similar mechanism occurs in human essential hypertension. Because the basic abnormality in spontaneous hypertension seems to be a diminished vascular growth, one may speculate that new antihypertensive drugs should promote vasoproliferation and in this way lower vascular resistance on the long run. Further investigations are required to explore this hypothesis.

In addition to morphometric studies of the microcirculation in SHR, this thesis focussed on neurogenic and hormonal influences on the microcirculation. An increased sympathetic drive has often been suggested to be one of the major events leading to arteriolar constriction and chronic hypertension (Abboud, 1982; 1984). Our data do not confirm this hypothesis, since no evidence was found for an early arteriolar constriction in SHR. This does not exclude an enhanced sympathetic activity in specific organs, e.g. the heart or kidneys. Heart rate was markedly elevated in the young SHR. An elevated heart rate at this age has been attributed to an enhanced activity of the sympathetic nervous system (Smith and Hutchins, 1979; Evenwel et al, 1983).

The studies, in which noradrenaline was applied via different vascular routes to adult rats, indicate marked differences in sensitivity along the vascular tree within one vascular bed as well as differences in sensitivity of different parallel coupled microvascular beds. This thesis was not designed to fully unravel pharmacological heterogeneity within the microcirculation. But so far, the experiments point at a potentially important direction for future cardiovascular pharmacological research.

The final aspect studied in this thesis was the influence of atrial natriuretic factors (ANF) on microcirculatory dynamics. Previous in vitro studies, using isolated blood vessels, and in vivo macrocirculatory studies in intact animals produced conflicting results as to the vascular effects of ANF. On the one hand, several authors (for references see chapter 1) have suggested a vasodilatory

effect of ANF, whereas others claim that ANF has no direct effects on resistance vessels. Our microcirculatory studies do not support the concept of ANF as a circulating vasodilatory substance. A second aspect of ANF activity, as derived from macrocirculatory studies, is its effects on body fluid dynamics. Hematocrit and plasma volume measurements in several species suggest that ANF may cause a shift of fluid from the intra to the extravascular fluid compartment. Our studies indicate that ANF does not cause albumin leakage in the mesenteric microcirculation. Although this does not exclude a transcapillary water flux, the data indicate that other mechanisms than transcapillary fluid shifts should be considered to explain the hematocrit changes observed by others.

In summary, this thesis has dealt with different physiological, pathological and pharmacological aspects of the microcirculation in a genetic animal model of hypertension. The studies indicate that the microcirculatory approach can provide new perspectives for studies on the pathophysiology and therapy of hypertension.

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10. SUMMARY

Spontaneous hypertension in rats is hemodynamically characterized by an increased peripheral resistance. However, the mechanism(s) by which the peripheral resistance is increased remain(s) unresolved. Control of vascular resistance occurs predominantly at the level of small arteries and arterioles. The aim of the present thesis was to investigate microvascular changes during the developmental phase as well as during the established phase of hypertension in a hypertensive model, the spontaneously hypertensive rat (SHR). Moreover, neurogenic and hormonal influences on the microcirculation were investigated to get more insight into the blood pressure regulation at this level of the circulation in SHR.

In the general introduction (chapter 1) current concepts on the aetiology of spontaneous hypertension are described. Although the nature of spontaneous hypertension is multifacetted, two main mechanisms can be distinguished. These concepts point towards the primary role of the kidneys or to primary vascular changes, initiating and maintaining the increased vascular resistance and thus blood pressure. Furthermore, a brief survey of the present major literature on microvascular changes in spontaneous hypertension is given. In section 1.7, the existing experimental microvascular data are synthetized into a hypothesis.

Chapter 2 describes the materials and the methods used in the experiments. Beside basic surgical techniques, different preparations for microvascular studies in anesthetized animals are described as well as a recently developed preparation which allows microcirculatory studies in conscious rats.

In chapter 3 the functional and structural vascular differences between hypertensive and normotensive control rats were studied in young rats (5-6 weeks). This age was chosen because after 5-6 weeks blood pressure starts to rise rapidly. Studies were performed on the cremaster muscle, which is a striated muscle and is morphologically considered to be a skeletal muscle, despite functional differences. Arteriolar diameters and vasodilating capacity were not different in

SHR and WKY control rats. In the SHR diminished arteriolar and capillary densities were found. This was also observed following maximal vasodilation suggesting a lower flow carrying capacity in the SHR.

In chapter 4 microvascular alterations in the rat dorsal cutaneous muscle were investigated. The study was performed in adult conscious rats. A comparison was made between arteriolar and venular diameters of spontaneously hypertensive and normotensive rats. Large arterioles in the SHR had diameters smaller than comparable vessels in the normotensive rats, while no differences were observed beween precapillary arterioles in both groups. Venular diameters were equal or slightly larger in SHR as compared to normotensive control animals.

An increased sympathetic drive has been often suggested to be one of the major events leading to arteriolar constriction and chronic hypertension. In chapter 5 the effects of sympathetic stimulation and exogenously applied adrenergic agents on microvascular dynamics are reported. The study was performed on the mesenteric microcirculation. In a number of experiments, the interrelationship between macro and microcirculatory events was studied. The results indicate that exogenous application of adrenergic and other vasoconstrictors or neurogenic stimuli produce an overall increase in total peripheral resistance with microvascular responses which differ both qualitatively and quantitatively, depending on the way in which the stimulus is applied.

In chapter 6 the regional hemodynamics in conscious rats and in rats anesthetized with pentobarbital or an alpha-chloralose-urethane mixture were compared. These anesthetics were chosen based on their frequent use in microvascular studies. The regional responses were studied following systemic application of differently acting vasopressor substances, like phenylephrine, noradrenaline and angiotensin II. The depressant activity of pentobarbital was more pronounced for the striated muscle bed while alpha-chloralose-urethane primarily affected the splanchnic bed.

Several authors have suggested a vasodilatory effect of ANF, whereas others claim that ANF has no direct effect on resistance vessels. Therefore, in chapter 7 the in-vivo effects of atriopeptin II (APII) on mesenteric microvessels were studied. Systemically applied APII caused a blood pressure decrease, parallelled by an arteriolar

constriction within the mesentery microvascular bed. No evidence was obtained for venular dilation, suggesting that larger vessels of other vascular beds are responsible for the blood pressure decrease. Local infusion of the drug into the target organ also did not induce arteriolar or venular dilation.

In chapter 8, the effect of another atrial natriuretic factor (102-126 hANF) on macromolecular leakage within the mesentery microcirculation was studied. Following systemic application of 102-126 hANF blood pressure decreased while no leakage occurred, indicating that fluid shifts (and thus a reduced effective circulating blood volume) are probably not responsible for the blood pressure decrease of 102-126 hANF.

SAMENVATTING

Hoewel uit vele studies blijkt dat spontane hypertensie gekenmerkt wordt door een verhoogde perifere vaatweerstand, zijn de mechanismen die hieraan ten grondslag liggen nog onbekend. De regulering van de vaatweerstand vindt voornamelijk plaats op het nivo van kleine arteriën en arteriolen.

In deze dissertatie werden de mikrovaskulaire veranderingen tijdens de ontwikkelingsfase en de stabiele fase van hypertensie bestudeerd in een diermodel, namelijk de spontaan hypertensieve rat (SHR). Bovendien werden neurogene en humorale faktoren bestudeerd, waarvan bekend is dat zij de tonus van mikrovaten kunnen beïnvloeden en daarmee de bloeddruk. Deze studies werden verricht om meer inzicht te krijgen in de regulering van de weerstand en daarmee van de bloeddruk bij spontane hypertensie.

In de algemene inleiding (hoofdstuk 1) worden de huidige opvattingen over de ziekteoorzaak van spontane hypertensie beschreven. Hoewel de oorzaak van spontane hypertensie vele facetten kent, zijn er twee belangrijke mechanismen beschreven. Het eerste konsept gaat uit van de rol van de nier, terwijl het tweede konsept vooral primaire veranderingen van het vaatstelsel verantwoordelijk acht voor zowel het initiëren als in stand houden van de verhoogde perifere weerstand en dus de bloeddruk. Verder wordt in dit hoofdstuk een kort overzicht gegeven van de belangrijkste literatuur op het gebied van mikrovaskulaire veranderingen tijdens spontane hypertensie. In paragraaf 1.7 worden de experimentele gegevens vervat in een hypothese.

In hoofdstuk 2 worden de materialen en methoden die in de verschillende experimenten gebruikt zijn beschreven. Naast basale chirurgische technieken wordt vooral aandacht besteed aan verschillende mikrovaskulaire preparaten bij verdoofde dieren. Ook wordt een preparaat beschreven dat recent ontwikkeld is en het mogelijk maakt om bij wakkere dieren mikrocirkulatoire studies te verrichten.

In hoofdstuk 3 worden de funktionele en strukturele vaskulaire verschillen tussen hypertensieve ratten en normotensieve kontroleratten beschreven. De studie werd verricht bij jonge ratten (5-6

weken). Deze leeftijd werd gekozen omdat het bekend is dat vanaf deze leeftijd de bloeddruk juist dan sterk gaat stijgen. Als preparaat werd gekozen voor de cremasterspier, een dwarsgestreepte spier. Zowel de arteriolaire diameter als het dilaterend vermogen is niet verschillend in SHR en WKY. In de spontaan hypertensieve rat werd een lagere arteriolaire en kapillaire dichtheid gevonden. Dit werd ook gevonden na maximale vaatverwijding. Het spiervaatbed van de spontaan hypertensieve rat is dan blijkbaar niet in staat om een extra aantal vaten te openen om daarmee de bloedstroom door het vaatbed te verhogen. Het onderliggende mechanisme verantwoordelijk voor de uiteindelijke verhoogde weerstand in de spontaan hypertensieve rat is een lagere arteriolaire en kapillaire dichtheid.

In hoofdstuk 4 worden mikrovaskulaire veranderingen beschreven in de huidspier in de rug van de rat. De studie werd verricht bij wakkere volwassen dieren. Er werd een vergelijking gemaakt tussen arteriolaire en venulaire diameters van spontaan hypertensieve en normotensieve ratten. Grote arteriolen verantwoordelijk voor een aanzienlijk deel van de perifere weerstand bij de spontaan hypertensieve rat toonden kleinere diameters dan overeenkomstige vaten bij normotensieve ratten. De diameters van prekapillaire arteriolen verschilden niet in beide groepen, terwijl de venulaire diameters in de spontaan hypertensieve rat iets groter waren dan overeenkomstige vaten bij normotensieve kontrole-dieren.

Men neemt aan dat een verhoogde sympathische tonus leidt tot een arteriolaire konstriktie en daarmee chronische hypertensie. In hoofdstuk 5 worden de effekten beschreven van sympathische stimulering en exogeen toegediende adrenerge stoffen op de hemodynamiek van de mikrocirkulatie. De studie werd uitgevoerd op het mesenterium van de rat. In een aantal experimenten werd de relatie beschreven tussen makro- en mikrocirkulatoire veranderingen. De resultaten laten zien dat na exogene toediening van adrenerge en andere vasokonstriktors of na neurogene stimulatie de totale perifere weerstand stijgt. Mikrovaskulaire effekten verschillen ten opzichte van de makrovaskulaire effekten afhankelijk van de wijze waarop gestimuleerd wordt. De effekten verschillen zowel in kwantitatief als kwalitatief opzicht.

In hoofdstuk 6 wordt de regionale hemodynamika van wakkere die-

ren vergeleken met die in dieren verdoofd met pentobarbital of met een alpha-chloralose-urethaan mengsel. Deze anesthetika werden gekozen op basis van hun frekwent gebruik voor mikrovaskulaire studies. De regionale effekten werden bestudeerd na systemische toediening van verschillend werkzame vasopressorsubstanties, zoals fenylefrine, noradrenaline en angiotensine II. Pentobarbital bleek selektief de reaktiviteit van het spierbed te verminderen, terwijl alfa-chloralose-urethaan meer de reaktiviteit van het darmbed verminderde.

Tenslotte werd de invloed bestudeerd van "atrial natriuretic factor" (ANF) op de mikrocirkulatoire hemodynamika. Litatuurgegevens zijn tegenstrijdig voor wat betreft de vaskulaire effekten van ANF. Zowel een (arteriolaire) dilatatie als konstriktie worden gerapporteerd. In hoofdstuk 7 worden de in-vivo effekten beschreven van atriopeptine II (APII) op de mikrovaten van het mesenterium. Systemisch toegediend APII verlaagde de bloeddruk, gepaard gaande met een arteriolaire konstriktie. Er werden geen direkte aanwijzingen gevonden voor venulaire dilatatie. Na lokale toediening van de stof in het doelorgaan trad arteriolaire konstriktie op, zonder venulaire dilatatie.

In hoofdstuk 8 worden de effekten beschreven van 102-126 hANF op de makromolekulaire lekkage van het mesenterium bij de rat. Na systemische toediening van de stof daalt de bloeddruk, terwijl geen makromolekulaire lekkage optreedt. Dit duidt er op dat een toegenomen perifeer watertransport in het darmbed naar het interstitium (en dus een afgenomen effektief cirkulerend bloedvolume) waarschijnlijk geen bijdrage levert aan het bloeddrukverlagende effekt van 102-126 hANF.

11. CURRICULUM VITAE

19 november 1956	Geboren te Heerlen
1969-1975	Atheneum-b gevolgd aan het Bernardinus Kollege te Heerlen. Tijdens middelbare schoolperiode na Nederlandse selektie deelname aan de "4th and 6th European Philips contest for young scientists and inventors" in respektievelijk 1972, Eindhoven, en 1974, Aken, West-Duitsland. Ontving in 1974 een prijs voor een studie getiteld "An environmental study on snails" (prof. dr. H.B.G. Casimir, destijds hoofd Philips Research).
1975-1981	Geneeskunde gestudeerd aan de Rijksuniversiteit Limburg te Maastricht Tijdens het 3e en 4e studiejaar werkzaam geweest als student-assistent in het laboratorium voor mikrocirkulatie (vakgroep Fysiologie; begeleider dr. G.J. Tangelder, hoofd prof. dr. R.S. Reneman). Het artsexamen werd met goed gevolg afgelegd in juni 1981.
1982-1987	In dienst getreden als wetenschappelijk assistent bij de vakgroep Farmakologie (hoofd prof. dr. H.A.J. Struyker Boudier). Tijdens de eerste 2 jaren werkzaam geweest op het gebied van de renale zenuwen en de betekenis daarvan bij het ontstaan van spontane hypertensie binnen het laboratorium voor dierexperimentele farmakologie (dr. J.F.M. Smits). Ontving voor dit werk in 1985 een prijs van de Revlon Health Care Group in de vorm van een reis-

beurs.
De experimenten waarop het huidige proefschrift gebaseerd is, zijn tot stand gekomen in de periode december 1983 - december 1986 en hoofdzakelijk uitgevoerd binnen het mikrocirkulatie-laboratorium (dr. D.W. Slaaf).
In 1986 gedurende een halfjaar werkzaam geweest in het mikrocirkulatielaboratorium van prof. dr. P.M. Hutchins en prof. dr. T.L. Smith te Winston-Salem N.C., U.S.A. (Wake Forest University, The Bowman Gray School of Medicine).

Vanaf 1987 — Assistent in opleiding op de afdeling Inwendige Geneeskunde van het Akademisch Ziekenhuis Maastricht (hoofd opleiding prof. dr. J. Flendrig).

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